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Dear Sir or Madam:

As a follow-up to a previous filing (attached) dated June 11, 1992, the International Isocyanate Institute Inc. (III) on behalf of its members (BASF Corporation, Dow Chemical Company, ICI Americas, Inc., Miles, Inc. and Olin Corporation) hereby submits a copy of the final report for the following study:

"Induction of respiratory hypersensitivity to diphenylmethane-4,4'-diisocyanate (MDI) in guinea pigs. Influence of route of exposure." III Project E-AB-82.

Very truly yours,

R. K. Rigger
Managing Director

RKR/sha
Enclosure

69 pgs.

FINAL REPORT

III PROJECT E-AB-82

TITLE ANIMAL MODEL FOR MDI

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ANIMAL MODEL FOR MDI

This Final Report is in three parts :

PART 1 Induction of respiratory hypersensitivity to diphenylmethane-4,4'-diisocyanate (MDI) in guinea pigs. Influence of route of exposure.

AUTHORS : N J Rattray, P A Botham, P M Hext, D R Woodcock, I Fielding, R J Dearman and I Kimber

This is the full text of a paper accepted for publication in 'Toxicology'.

PART 2 Appendix 1. Breathing Pattern Measurements.

This appendix gives additional data on the studies described in Part 1.

PART 3 Appendix 2. Breathing rate pattern changes in guinea pigs sensitised to MDI and challenged with MDI aerosols

This appendix gives typical examples of the new data of the breathing pattern rate and pattern changes found in Project E-AB-82.

III PROJECT E-AB-82

11096

ANIMAL MODEL FOR MDI

PART 1

INDUCTION OF RESPIRATORY HYPERSENSITIVITY TO DIPHENYLMETHANE-4,4'-DIISOCYANATE (MDI) IN GUINEA PIGS. INFLUENCE OF ROUTE OF EXPOSURE

AUTHORS

N J Rattray, P A Botham, P M Hext,
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and I Kimber

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Induction of respiratory hypersensitivity to diphenylmethane-4,4'-diisocyanate (MDI) in guinea pigs. Influence of route of exposure

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Key words: Respiratory hypersensitivity, inhalation exposure, diphenylmethane-4,4'-diisocyanate, guinea pigs.

Summary

The induction of respiratory sensitization in guinea pigs to diphenylmethane-4,4'-diisocyanate (MDI), a known human respiratory allergen, has been investigated and different routes of exposure compared. Guinea pigs were exposed to MDI by intradermal injection, by topical application or by inhalation. Pulmonary hypersensitivity was measured subsequently as a function of changes in respiratory rate following challenge with atmospheres containing MDI. In addition contact hypersensitivity was measured by topical challenge and antibody responses evaluated by enzyme-linked immunosorbent assay (ELISA) and passive cutaneous anaphylaxis (PCA). Attempts to sensitize guinea pigs by inhalation exposure to MDI were unsuccessful. Antibody responses and contact sensitization were both infrequent and low grade, and no animals exhibited pulmonary responses following challenge with atmospheric MDI. In contrast, sensitization by either intradermal injection or topical application of MDI induced antibody responses in the majority of animals. Moreover, a proportion of animals in each case exhibited pulmonary responses following subsequent inhalation challenge.

These data indicate that the route of exposure influences markedly the effectiveness of sensitization to respiratory allergens such as MDI and that skin contact may be an important cause of occupational respiratory allergy.

Introduction

A variety of chemicals, including some acid anhydrides [1-3], reactive dyes [4-6], platinum salts [7,8] and diisocyanates [9-11], are known to cause occupational respiratory allergy, associated frequently with the presence of specific IgE antibody.

The guinea pig has been used extensively to model respiratory hypersensitivity reactions induced by chemicals. It has been shown that inhalation exposure of guinea pigs to allergens in the form of either the free chemical or a hapten-protein conjugate results in respiratory hypersensitivity when animals are challenged subsequently with atmospheres containing the relevant chemical conjugate [12-15].

The acute-onset of respiratory hypersensitivity is a consequence of homocytotropic antibody-induced vasodilation and bronchoconstriction. There is no a priori reason to believe that the induction of homocytotropic antibody responses and sensitization for respiratory allergy will be caused solely by inhalation exposure. Indeed, there is evidence that occupational respiratory hypersensitivity may result from dermal exposure to chemical allergens following industrial spillage or splashing [16]. This also can be modelled in guinea pigs. A number of reports demonstrate that respiratory hypersensitivity reactions can be elicited by inhalation challenge, with free or protein-bound chemical, of guinea pigs sensitized previously by either topical or intradermal exposure to the free chemical [17-19].

There is little information available regarding the relative effectiveness of these different routes of exposure for respiratory sensitization. In

the present study we have examined the ability of diphenylmethane-4,4'-diisocyanate (MDI) to induce respiratory hypersensitivity in guinea pigs when administered by routes other than inhalation. MDI, like toluene isocyanate (TDI), is an aromatic diisocyanate which has been used widely in the manufacture of polyurethanes and which is known to have the potential to cause occupational respiratory hypersensitivity [20-22]. We have measured serological responses and respiratory and dermal hypersensitivity reactions following exposure of guinea pigs to various concentrations of MDI by either intradermal or topical routes. In addition, in a single experiment the same parameters have been measured following inhalation exposure to a single concentration of MDI.

Materials and Methods

Animals

Female Dunkin-Hartley albino guinea pigs (Harlan Porcellus Animal Breeding, Sussex, UK) with an initial weight range of approximately 250-300g were used in all studies. Animals were acclimatized for a period of at least 10 days, randomized and housed individually. Guinea pigs were allowed food and water ad libitum except during inhalation exposure periods.

Chemical and hapten-protein conjugate

Monomeric diphenylmethane-4,4'-diisocyanate (MDI) was obtained from ICI Polyurethanes, Everslaan, Belgium.

Conjugates of MDI with guinea pig serum albumin (GPSA; Sigma Chemical Co., St. Louis, MO) were prepared as follows. GPSA (200mg) was dissolved in 20ml borate buffer (pH 9.4). MDI (60mg) was added and the solution stirred at 4°C for 30 minutes. The solution was dialyzed successively against phosphate-buffered saline (PBS; pH 7.2) and distilled water. The conjugate was lyophilized and stored at -20°C until use.

The degree of substitution of MDI conjugates was assessed using a method based upon determination of free amino groups by reaction with 2,4,6-trinitrobenzene sulphonic acid (TNBS) [23]. Conjugates and GPSA at 1mg/ml in borate buffer (pH 9.3) were incubated for 20 minutes at room temperature in the presence of 0.03M TNBS. The optical density at 420nm

was measured. GPSA has approximately 30 readily available hapten-binding sites per molecule. Hence the degree of substitution (mol/mol) was calculated according to the formula:-

$$\text{Substitution} = \left(1 - \frac{\text{OD sample}}{\text{OD GPSA}}\right) \times 30$$

MDI-conjugates were found to have substitution ratios of approximately 20:1 (moles hapten:moles protein).

Sensitization

(i) **Topical sensitization**

Groups of guinea pigs received a single topical application to the shaved scapular region of 400μl of various concentrations of MDI in corn oil, or an equal volume of corn oil alone. Application sites were occluded for 6hr.

(ii) **Intradermal sensitization**

Guinea pigs received a single intradermal injection of 100μl of various concentrations of MDI in corn oil, or of an equal volume of corn oil alone.

(iii) **Inhalation sensitization**

Guinea pigs received 5 consecutive daily exposures (nose only) for 3 hours to atmospheres containing between 19.4 and 23.7mg/m^3 MDI. Control animals received identical exposure to dry air.

Challenge

Guinea pigs were challenged 21 days following the initiation of sensitization by inhalation exposure to atmospheres containing various concentrations of MDI.

As described previously by Karol et al [12], challenge-induced respiratory hypersensitivity reactions in guinea pigs are characterized by an increase in respiration rate and a decrease in tidal volume (rapid shallow breathing) which may progress to a slow gasping breathing pattern reflecting severe bronchoconstriction. Respiratory rate monitoring was accomplished by using individual whole-body plethysmograph tubes which also permitted nose-only exposure to atmospheres generated into perspex exposure chambers of 28cm diameter and an internal volume of approximately 40 litres. Airflow through the chambers varied according to the experimental procedure but was always in excess of 12 air changes per hour. Pressure plethysmography was conducted using a system comprising pressure transducers linked to a microcomputer running the Respiratory Analysis Programme (RASP) (Physiologic Ltd, Newbury, Berks, UK). Each pressure transducer was linked to the rear of the individual whole-body plethysmographs and up to 8 could be accommodated by the system. The pressure changes within the plethysmograph due to animal respiration were detected via the pressure transducer, amplified and analysed to provide respiratory rate.

Typically, the challenge regimen comprised a settling period, usually of 15 minutes, followed by a period of at least 10 minutes to establish a

stable base line rate of respiration. Challenge with atmospheres of MDI was performed for 15 minutes and respiration rate monitored for an additional 15 minutes after removal from the challenge atmosphere.

The concentrations employed for challenge exposure were selected on the basis of preliminary studies in which guinea pigs were exposed to increasing concentrations of atmospheric MDI in order to determine the threshold for induction of sensory irritation, measured as a function of reduced respiratory rate. Such studies were performed to ensure that responses observed in sensitized animals were not attributable to pulmonary irritation. The selection of appropriate challenge concentrations was confirmed using relevant control groups (non-sensitized guinea pigs) in each experiment.

Pulmonary responses were recorded as either positive or negative. A positive response was defined as either a rapid decrease (to 70% or less), or an increase (to 130% or greater) in respiration rate relative to pre-challenge values during the 15 minute challenge period. Changes in respiration rate during the challenge period of between 71% and 129% of the mean pre-challenge values were defined as negative responses.

Atmosphere generation and analysis

Atmospheres of MDI, used for both inhalation sensitization and challenge, were generated as follows. Pre-warmed air was passed over the surface of MDI maintained at 65°C to create a saturated vapour of the chemical. The MDI vapour was condensed by cooling to form an aerosol which was adjusted with air to provide the appropriate atmospheric concentration.

Particulate concentrations were measured gravimetrically using VM-1 25mm open-faced filters (Gelman, Northampton, UK). Particle size distribution was determined using a cascade impactor (Marple Cascade Impactor; Schaeffer Instruments, Wantage, UK). All atmospheres were sampled in the breathing zone of guinea pigs.

Serological analyses

Blood was drawn from guinea pigs by cardiac puncture 18 days following the initiation of exposure. Serum was prepared and stored at -20°C until use.

(i) Passive cutaneous anaphylaxis (PCA)

Serum from guinea pigs exposed previously to MDI and from control animals, was diluted 1:2 with physiological saline. An aliquot (100μl) of diluted serum was injected intradermally into the shorn flanks of naive guinea pigs. Six samples were injected into each recipient. Tests were performed either 6h or 6 days later to measure IgG1 and IgE homocytotropic antibody, respectively.

Animals were injected intravenously with 500μl of sterile physiological saline containing 2.5mg of MDI-GPSA conjugate and 5mg of Evans Blue dye. Cutaneous reactions were evaluated after 30 minutes and positive responses defined as those which resulted in a local blue lesion of 3mm or greater diameter.

(ii) Enzyme-linked immunosorbent assay (ELISA)

Plastic microtitre plates (Nunc Immunoplate type II, Nunc, Copenhagen, Denmark) were coated with 5μg/ml of MDI-GPSA conjugate in 0.05M sodium carbonate/bicarbonate buffer (pH 9.6) by overnight incubation at 4°C. Various dilutions of guinea pig serum were added (100μl aliquots) and the plates incubated for 30 minutes at 37°C. Plates were washed (x3) in PBS containing 0.05% Tween 20 (PBS-Tween) and 100μl of rabbit anti-guinea pig IgG1 (Miles Scientific, Slough, UK), diluted 1:2500 in PBS-Tween added to each well. Plates were again incubated for 30 minutes at 37°C and washed prior to addition of a peroxidase-labelled goat anti-rabbit IgG (Miles Scientific), diluted 1:5000 with PBS-Tween. Following a further 30 minutes incubation at 37°C the plates were again washed and substrate (o-phenylenediamine) added. Reactions were terminated after 10 minutes by addition of 0.5M citric acid. Absorbance at 450nm was measured using an automatic reader (Multiskan, Flow Laboratories, Irvine, Ayrshire, UK). Results are expressed as the reciprocal of the highest dilution of serum which resulted in an OD_{450} of twice the reagent background.

Dermal hypersensitivity reactions

Dermal hypersensitivity was assessed 22 days following the initiation of exposure, using a modification of the challenge procedure described by Magnusson and Kligman [24]. Briefly, guinea pigs were challenged on the shaved flanks with 100μl of a non-irritant (3%) concentration of MDI. The application site was occluded and the dressing left in place for 24h. Reactions were assessed 24 and 48h following removal of the dressing and scored as follows: 0 (no reaction), 1 (scattered mild redness), 2 (moderate diffuse redness) or 3 (intense redness or swelling).

Results

Intradermal sensitization

In initial experiments groups of guinea pigs were exposed by intradermal injection to various concentrations (0.0003 to 0.3%) of MDI; a route of exposure shown previously in this Laboratory to induce in guinea pigs pulmonary hypersensitivity to trimellitic anhydride [18]. Twenty one days following sensitization all guinea pigs were exposed to atmospheric concentrations of MDI of between 27.6 and 36.5mg/m^3. Treatment with both 0.03% and 0.3% intradermal MDI resulted in pulmonary hypersensitivity with, in each case, 5 of 8 test animals exhibiting marked changes in respiratory rate following inhalation challenge (Table I). Only 1 of 8 animals which received 0.003% MDI and no animals which had been treated with 0.0003% MDI or with vehicle (corn oil) alone exhibited changes in respiratory rate (Table I). Blood was drawn from all animals 18 days following exposure and the presence of IgG1 anti-MDI antibody in serum measured by ELISA. As the results summarized in Table I indicate, no specific antibody was found in serum from control animals which had received vehicle alone or in animals treated with 0.0003% MDI. Two of 8 guinea pigs sensitized with 0.003% MDI and all animals sensitized with either 0.03% or 0.3% MDI exhibited IgG1 anti-MDI antibody. High titre (1:2560 or greater) antibody was found in the serum of all guinea pigs exposed to 0.3% MDI. There was, however, no strong correlation between the presence of IgG1 anti-MDI antibody in serum and the elicitation of significant changes in respiratory rate following inhalation challenge. Thus, a single animal in the group treated with 0.003% MDI exhibited relatively high titre (1:640) antibody, but failed to

display a positive response in terms of respiratory rate change following challenge. Moreover, in the group exposed to 0.03% MDI, only 1 of the 2 guinea pigs which were found to have the highest titre antibody (≥1:10240) exhibited positive respiratory rate changes after challenge.

The same serum samples were used also to measure PCA. In this series of experiments only 6hr reactions were measured, a time point at which mast cell-bound IgG1 is detected primarily. Serum from 1 of 8 and 3 of 8 guinea pigs in the groups sensitized respectively with 0.03% and 0.3% MDI, induced positive PCA responses. Again there was no absolute correlation with challenge-induced changes in respiratory rate. One guinea pig sensitized with 0.3% MDI, serum from which induced PCA, failed to exhibit a significant alteration in respiratory rate following inhalation challenge. Although in all other instances a PCA reaction was associated with a positive challenge response, it is apparent that a significant challenge-induced respiratory rate change is not necessarily associated with PCA activity (Table I).

Dermal hypersensitivity was examined 22 days following sensitization. Following topical challenge with 3% MDI none of the control guinea pigs exposed previously to vehicle alone exhibited contact hypersensitivity reactions. In animals sensitized with MDI only sporadic, and usually low grade, challenge reactions were observed. Interestingly, in the group of guinea pigs sensitized intradermally with the highest concentration of MDI (0.3%) no challenge reactions were observed at 24 hours and only a single weak reaction at 48 hours (Table II).

Topical sensitization

Groups of guinea pigs were exposed topically, under occlusion, to various concentrations (10%, 30% and 100%) of MDI, or to vehicle alone. The elicitation of pulmonary hypersensitivity was measured 21 days following treatment by inhalation challenge with atmospheres containing between 25.9 and 36.4mg/m^3 MDI. Control animals exposed previously to vehicle alone failed to develop pulmonary responses following challenge. In the groups sensitized with 10% or 30% MDI positive respiratory rate changes were in both cases recorded for 2 of 8 animals. In guinea pigs treated with 100% MDI, 3 of 7 animals tested exhibited challenge-induced respiratory rate changes (Table III).

As determined by analysis (ELISA) of serum prepared from animals 18 days following sensitization, only 1 of 8 guinea pigs treated with 10% MDI was found to have elicited an antibody response. In guinea pigs sensitized with 30% or 100% MDI there was evidence for an anti-hapten antibody response in 5 of 8 and 7 of 8 test animals, respectively. No antibody was detected in serum from vehicle-treated controls (Table III). Here again there was no obvious correlation between the titre of IgG1 anti-hapten antibody as determined by ELISA and challenge-induced respiratory rate changes. Although 1 of 2 guinea pigs sensitized with 30% MDI, and which exhibited pulmonary responses, was found to have the highest titre antibody (1:2560), several animals in the group treated with 100% MDI and which were shown to have the same antibody titre, failed to display significant changes in respiratory rate following challenge.

Serum of 2 animals each from groups sensitized with 30% or 100% MDI exhibited activity in a 6hr PCA assay (Table III). In each of these cases a 6 day PCA assay was negative (data not presented). In guinea pigs treated with 30% MDI only 1 of 2 animals with PCA-positive sera exhibited a pulmonary response. Of the 2 animals in the group sensitized with 100% MDI which were found to have PCA activity, 1 displayed a challenge-induced pulmonary response, the second was not tested.

Topical challenge of guinea pigs 22 days following the initiation of treatment induced dermal reactions in greater than 50% of all MDI-sensitized animals. No contact reactions were observed following challenge of vehicle-treated controls (Table IV). There was no apparent correlation between the incidence and severity of dermal hypersensitivity with either the elicitation of pulmonary responses or antibody titre.

Inhalation sensitization

Guinea pigs were exposed to atmospheres containing between 19.4 and 23.7mg/m^3 MDI. Control animals received dry air alone. Pulmonary responses were measured 21 days following the initiation of sensitization by inhalation challenge of all animals with atmospheres containing between 34.6 and 44.1mg/m^3 MDI.

A significant change in respiratory rate was observed in only a single vehicle-treated control animal. Guinea pigs exposed previously to atmospheric MDI failed to develop pulmonary responses (Table V). As determined by ELISA, 18 days following the initiation of inhalation

sensitization with MDI, only 3 of 15 animals were found to have serum anti-hapten antibody, and this was of low titre (1:160 or less). No antibody was detected in serum prepared from control animals and serum from neither sensitized nor control guinea pig was active in a 6hr PCA assay (Table V). Dermal hypersensitivity was measured 22 days following treatment by topical challenge with 3% MDI. No cutaneous reactions were observed in control animals. Grade 1 skin reactions were recorded for 2 of 16 test animals at 24hr and for 3 of 16 animals at 48hr (data not presented).

Discussion

The data presented here demonstrate clearly that MDI, a known human respiratory allergen, is able to induce respiratory hypersensitivity in guinea pigs when administered by routes other than inhalation exposure. As such they serve to confirm and extend the results of previous investigations in which exposure of guinea pigs to intradermal trimellitic anhydride (TMA) [18,19] or topical TDI [17] has been shown to cause respiratory sensitization. In the single experiment reported here, inhalation exposure of guinea pigs to unconjugated MDI failed to induce respiratory sensitivity. The results of other studies have found inhalation exposure to certain chemical respiratory allergens ineffective, or at least less effective than intradermal injection, for sensitization [14,19]. As many studies in which symptoms of respiratory hypersensitivity have been provoked successfully in guinea pigs sensitized previously with the free chemical have employed the relevant hapten-protein conjugate for challenge, the failure, in the present investigation, to elicit pulmonary responses with free chemical is perhaps not surprising. More unexpected was the very weak immunogenicity of inhaled MDI, with evidence only for low titre antibody and/or low grade contact sensitization in a minority of exposed animals. It is instructive to consider these data in the context of previous studies in which the chemical respiratory allergens TMA and YMX4R, a reactive dye, were examined and compared with TDI. Inhalation exposure of guinea pigs to free TDI was found to induce specific sensitization and to result in pulmonary reactions when animals were challenged subsequently with atmospheres containing a TDI-GPSA conjugate [14]. Under the same conditions, guinea pigs exposed by inhalation to TMA and YMX4R failed to exhibit changes in respiratory rate following challenge

with the relevant hapten-protein conjugates [14]. It was found, however, that many of the guinea pigs exposed to TMA had serum IgG1 anti-hapten antibody and that some had IgE antibody also. Similarly, YMX4R induced specific IgG1 antibody and, in a proportion of exposed animals, a transient IgE response [14]. The failure of inhaled MDI in the present study to induce a significant humoral or cell-mediated immune response could be considered to be attributable partly to the disposition of the chemical within the respiratory tract. The disposition of inhaled aerosols in experimental animals is a function largely of particle size [25]. In the present study the mean particle size (mass mean aerodynamic diameter) of atmospheric MDI used for inhalation sensitization was approximately 1.5μm. In the studies quoted above [14], where there was evidence for IgG1 and IgE antibody following inhalation exposure to TMA, the MMAD of atmospheric TMA was found to be in the range of 3.6 to 3.8μm. It may be concluded therefore, that the inability of MDI to provoke an antibody response is unlikely to be due exclusively to inappropriate disposition within the respiratory tract.

Another possibility is that, as the result of local metabolism, atmospheric concentrations of MDI do not reflect delivered dose to the respiratory tract-associated lymphoid tissue. Such has been proposed previously to explain the comparatively weak immunogenicity of inhaled 2,4-dinitrochlorobenzene in mice [26]. As MDI is highly reactive it is possible also that the inhaled chemical associates with macromolecules in such a way as to form protein conjugates which are non-immunogenic. Alternatively, MDI may in fact reach the local lymphoid tissue but interact with the immune system to cause active down-regulation of humoral and cell-

mediated immunity. Precedents exist. There is clear evidence that inhalation exposure of rodents to protein antigens, such as ovalbumin, causes an active and specific suppression of immune function, and in particular of IgE responses [27-30]. It is apparent also that inhalation exposure of animals to chemical respiratory allergens can result in antigen-specific suppression of subsequent IgE responses [31] and of contact sensitization [32]. The possibility exists, therefore, that in the present investigations inhalation exposure of guinea pigs to MDI has resulted in a similar specific down-regulation of immune function. It is important to emphasize that in the investigations reported here inhalation sensitization was attempted with only a single concentration of MDI. It can not be assumed from these data that MDI is unable always to induce respiratory sensitization when administered via inhalation. It is possible that other exposure concentrations would have been effective.

Irrespective of the mechanisms responsible for the weak immunogenicity of inhaled MDI in the present study, it is clear from the data presented here that intradermal injection or topical application of the same chemical induces in a proportion of guinea pigs specific antibody responses and pulmonary hypersensitivity. The differences in immunogenicity observed clearly reflect variation in exposure route rather than the concentration of MDI used for sensitization. Intradermal injection of, for instance, 0.03% MDI, which elicited pulmonary responses in 5 of 8 guinea pigs and antibody production in all guinea pigs, corresponds to a total applied dose of 30μg. The minute volume of a guinea pig is approximately 200ml/minute. It can be calculated that guinea pigs exposed to atmospheric concentrations of MDI of between 19.4 and 23.7mg/m^3 (average 22.7mg/m^3) inhaled approximately

4.5μg/minute of the aerosol which is equivalent to 4mg in total during 5 consecutive daily 3 hour exposures. A particle size distribution of between 1 and 4μm has been shown to result in 50% to 90% deposition in the respiratory tract [25], suggesting a cumulative intake of between approximately 2 and 3.6mg in the study described here. It must be recognized, however, that in these studies inhalation exposure to only a single concentration was examined. It is entirely possible that lower atmospheric concentrations of the chemical, resulting in a lower delivered dose, might prove effective at inducing respiratory sensitization.

The reasons for the apparent lack of correlation between serum antibody and pulmonary responsiveness in guinea pigs sensitized by intradermal injection or topical application are unclear. It is possible however, that in some instances, changes in respiratory rate (as measured here) are of insufficient sensitivity to detect smaller, but biologically relevant, alterations in respiratory function.

The ability of topical exposure to cause respiratory sensitization is of considerable interest, particularly in the context of occupational medicine and the identification of appropriate operating practices and hygiene standards. There is, of course, no reason to suppose that cutaneous contact with chemical respiratory allergens will not result in the appearance of homocytotropic antibody and in pulmonary hypersensitivity following subsequent exposure to atmospheres containing the same chemical. Indeed it has been shown recently in mice that topical exposure to chemical respiratory allergens results in IgE antibody production [33-36], the active and specific sensitization of mast cells <u>in vivo</u> [37] and immediate-type

dermal hypersensitivity reactions following subsequent topical challenge [38]. The results contained within this report confirm that routes of exposure other than inhalation may induce respiratory sensitization to chemicals and suggest that skin contact with respiratory allergens may represent an important occupational hazard.

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TABLE I

SUMMARY OF PULMONARY HYPERSENSITIVITY AND ANTIBODY RESPONSES FOLLOWING INTRADERMAL SENSITIZATION OF GUINEA PIGS TO MDI

Group	MDI % (w/v)	Pulmonary responses	IgG1 serum antibody (ELISA) Titre (no. of responses)						PCA (6h)
			<10	40	160	640	2560	≥10240	
1	0	0/8	8						0/8
2	0.0003	0/6	8						0/8
3	0.003	1/8	6	1		1			0/8
4	0.03	5/8			1	4	1	2	1/8
5	0.3	5/8					4	4	3/8

Guinea pigs were exposed to various concentrations of MDI, or to vehicle (corn oil) alone, by a single intradermal injection. Serum was prepared from blood drawn 18 days following exposure. Pulmonary responses were measured 21 days following treatment by inhalation exposure to atmospheres containing between 27.6 and 36.5mg/m^3 MDI (Group 1, 30.3mg/m^3; Group 2, 27.6mg/m^3; Group 3, 35.0mg/m^3; Group 4, 36.5mg/m^3; Group 5, 35.2mg/m^3).

TABLE II

DERMAL HYPERSENSITIVITY RESPONSES FOLLOWING INTRADERMAL SENSITIZATION OF GUINEA PIGS TO MDI: A SUMMARY

Group	MDI % (w/v)	24h (no. of responders)					48h (no. of responders)				
		ND	0	1	2	3	ND	0	1	2	3
1	0		8					8			
2	0.0003	3	3	1	1		3	4	1		
3	0.003		5	3				6	1	1	
4	0.03	2	6				2	6			
5	0.3		8					7	1		

Guinea pigs were exposed to various concentrations of MDI, or to vehicle (corn oil) alone, by a single intradermal injection. Dermal hypersensitivity was measured 22 days following exposure by topical challenge with 3% MDI.

ND = not determined

TABLE III

SUMMARY OF PULMONARY HYPERSENSITIVITY AND ANTIBODY RESPONSES FOLLOWING TOPICAL SENSITIZATION OF GUINEA PIGS TO MDI

Group	MDI % (w/v)	Pulmonary responses	IgG1 serum antibody (ELISA) Titre (no. of responses)					PCA (6h)
			<10	40	160	640	2560	
1	0	0/8	8					0/8
2	10	2/8	7		1			0/8
3	30	2/8	3	1	1	2	1	2/8
4	100	3/7	1			1	6	2/8

Guinea pigs were exposed to various concentrations of MDI, or to vehicle (corn oil) alone, by a single topical application. Serum was prepared from blood drawn 18 days following exposure. Pulmonary responses were measured 21 days following treatment by inhalation exposure to atmospheres containing between 25.9 and 36.4mg/m^3 MDI (Group 1, 30.8mg/m^3; Group 2, 25.9mg/m^3; Group 3, 29.2mg/m^3; Group 4, 36.4mg/m^3).

TABLE IV

DERMAL HYPERSENSITIVITY RESPONSES FOLLOWING TOPICAL SENSITIZATION OF GUINEA PIGS TO MDI: A SUMMARY

Group	MDI % (w/v)	24h (no. of responders)					48h (no. of responders)				
		ND	0	1	2	3	ND	0	1	2	3
1	0		8					8			
2	10		3	5				3	5		
3	30		1	5	2			3	3		2
4	100	1	1	1	5		1	2	3	1	1

Guinea pigs were exposed to various concentrations of MDI, or to vehicle (corn oil) alone, by a single topical application. Dermal hypersensitivity was measured 22 days following exposure by topical challenge with 3% MDI.

ND = not determined

TABLE V

SUMMARY OF PULMONARY HYPERSENSITIVITY AND ANTIBODY RESPONSES FOLLOWING INHALATION SENSITIZATION OF GUINEA PIGS TO MDI

Group	MDI mg/m^3	Pulmonary responses	IgG1 serum antibody (ELISA) Titre (no. of responses)			PCA (6h)
			<10	40	160	
1	0	1/7	8			0/8
2	19.4-23.7	0/16	13	2	1	0/16

Guinea pigs were exposed to atmospheres containing between 19.4 and 23.7mg/m^3 MDI, or to dry air alone. Inhalation exposure was performed for 3 hours on each of 5 consecutive days. Serum was prepared from blood drawn 18 days following the initiation of exposure. Pulmonary responses were measured 21 days following the initiation of treatment by inhalation exposure to atmospheres containing between 34.6 and 44.1mg/m^3 MDI (Group 1, either 34.6 or 44.1mg/m^3; Group 2, 34.6, 43.4 or 44.1mg/m^3).

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ANIMAL MODEL FOR MDI

PART 2

APPENDIX 1

BREATHING PATTERN MEASUREMENTS

APPENDIX

BREATHING PATTERN MEASUREMENTS

In the experiments described, breathing pattern, in addition to alterations in respiratory rate, was measured. The rationale was that the elicitation of pulmonary reactions in previously sensitized guinea pigs may cause perturbations in breathing pattern independently, or in the absence, of substantial alterations in respiratory rate.

Breathing pattern data were evaluated using a Respiratory Analysis Programme (RASP). Breathing patterns for individual animals were displayed continuously on a monitor screen and recordings made at 8 second intervals during the stabilization, challenge and recovery periods. Normal breathing pattern is described by a smooth sine-wave form, with the inspiration and expiration phases being of approximately equal length. Significant changes in wave form resulting from challenge were classified as being indicative of a respiratory hypersensitivity reaction.

TABLES I and IA

Untreated control animals (group 1) displayed neither changes in respiratory rate nor abnormal breathing patterns following inhalation challenge with an atmosphere of MDI. No guinea pigs sensitized intradermally with 0.0003% MDI (group 2) showed changes in respiratory rate following challenge and only 1/6 guinea pigs displayed an abnormal breathing pattern. In group 3 (guinea pigs sensitized intradermally with 0.003% MDI) 5/8 animals showed challenge-induced changes in breathing pattern, while only 1/8 exhibited alterations in respiratory rate. In the

highest sensitization dose groups (group 4, 0.03% MDI and group 5, 0.3% MDI) 5/8 guinea pigs were found to exhibit changes in respiratory rate. In the same groups 4/8 and 8/8 animals, respectively displayed abnormal breathing patterns. Using the criteria for positive responses employed here, it is clear that, in some instances, abnormal breathing patterns were observed in the absence of substantial changes in respiratory rate. Such differences are most obvious in group 3. It is apparent also, however, that a substantial change in respiratory rate may be observed in the absence of an abnormal breathing pattern (animals no 3 and 4, group 4).

A general association exists between increasing IgG1 anti-MDI antibody titre and the frequency of pulmonary responses following challenge. However, such associations are not invariable as is clear when responses provoked in individual animals are examined. Thus, antibody titres of as high as 1/2560 and 1/10240 are not always indicative of a pulmonary reaction as defined here.

TABLES III and IIIA

Here again there is no evidence for pulmonary responses in untreated control animals challenged by inhalation exposure to MLI. In groups 2 and 3 (guinea pigs sensitized topically with 10% and 30% MDI, respectively) 2/8 animals exhibited challenge-induced changes in respiratory rate. In the same groups, 1/8 guinea pigs in each case exhibited abnormal breathing patterns. In the highest dose group (topical exposure to 100% MDI), 3/7 guinea pigs showed changes in respiratory rate and 5/7 guinea pigs abnormal breathing patterns.

TABLES V and VA

No control animals exhibited abnormal breathing patterns following challenge and only 1/7 animals was found to have an altered respiratory rate. No guinea pigs sensitized by inhalation exposure to atmospheres of MDI exhibited pulmonary reactions when challenged by the same route.

In summary, incorporation of data derived from measurement of challenge-induced changes in breathing pattern does not influence or alter the conclusions drawn from analysis of respiratory rate alone. These conclusions are discussed in detail in the main paper. Neither does examination of breathing pattern serve to clarify the relationship between IgG1 anti-hapten antibody titre and the elicitation of pulmonary reactions in previously sensitized guinea pigs. On the basis of the studies performed and the data presented here it is not possible to draw firm conclusions about the relative merits and sensitivity of respiratory rate and breathing pattern measurements.

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TABLE IA

PULMONARY REACTIONS AND ANTIBODY RESPONSES FOLLOWING INTRADERMAL SENSITIZATION OF GUINEA PIGS TO MDI

Animal	Group 1 (0)			Group 2 (0.0003)			Group 3 (0.003)			Group 4 (0.03)			Group 5 (0.3)		
	R[1]	T[2]	Ab[3]	R	T	Ab	R	T	Ab	R	T	Ab	R	T	Ab
1	-	-	<10	-	-	<10	-	+	10	-	-	640	+	+	10240
2	-	-	<10	-	-	<10	-	+	40	-	-	10240	-	+	2560
3	-	-	<10	-	-	<10	-	-	<10	+	-	640	+	+	10240
4	-	-	<10	-	+	<10	-	+	<10	+	-	10240	-	+	10240
5	-	-	<10	-	-	<10	-	+	640	+	+	2560	+	+	10240
6	-	-	<10	NT[4]	NT	<10	+	+	<10	+	+	640	+	+	2560
7	-	-	<10	-	-	<10	-	+	<10	-	+	160	+	+	2560
8	-	-	<10	NT	NT	<10	-	-	<10	+	+	640	-	+	2560
Total	0/8	0/8		0/6	1/6		1/8	5/8		5/8	4/8		5/8	8/8	

[1] Respiratory rate; [2] Respiratory trace, breathing pattern; [3] Reciprocal IgG1 titre (ELISA); [4] Not tested

TABLE IIIA

PULMONARY REACTIONS AND ANTIBODY RESPONSES FOLLOWING TOPICAL SENSITIZATION OF GUINEA PIGS TO MDI

Animal	Group 1 (0)			Group 2 (10)			Group 3 (30)			Group 4 (100)		
	R[1]	T[2]	Ab[3]	R	T	Ab	R	T	Ab	R	T	Ab
1	-	-	<10	-	-	<10	-	-	640	+	+	2560
2	-	-	<10	-	-	<10	-	-	<10	-	+	2560
3	-	-	<10	-	-	<10	-	-	160	+	-	2560
4	-	-	<10	-	-	<10	+	-	40	-	+	2560
5	-	-	<10	+	+	160	-	-	<10	+	+	2560
6	-	-	<10	-	-	<10	-	-	640	-	-	<10
7	-	-	<10	+	-	<10	-	-	<10	-	+	2560
8	-	-	<10	-	-	<10	+	+	2560	NT[4]	NT	640
Total	0/8	0/8		2/8	1/8		2/8	1/8		3/7	5/7	

[1] Respiratory rate; [2] Respiratory trace, breathing pattern; [3] Reciprocal IgG1 titre (ELISA); [4] Not tested

TABLE VA

PULMONARY REACTIONS AND ANTIBODY RESPONSES FOLLOWING INHALATION SENSITIZATION OF GUINEA PIGS TO MDI

Animal	Group 1 (0)			Group 2 (19.4-23.7)		
	R[1]	T[2]	Ab[3]	R	T	Ab
1	-	-	<10	-	-	<10
2	NT[4]	NT	<10	-	-	<10
3	-	-	<10	-	-	<10
4	-	-	<10	-	-	40
5	+	-	<10	-	-	<10
6	-	-	<10	-	-	160
7	-	-	<10	-	-	<10
8	-	-	<10	-	-	<10
9				-	-	<10
10				-	-	<10
11				-	-	<10
12				-	-	<10
13				-	-	<10
14				-	-	40
15				-	-	<10
16				-	-	<10
Total	1/7	0/7		0/16	0/16	

[1] Respiratory rate; [2] Respiratory trace, breathing pattern; [3] Reciprocal IgG1 titre (ELISA); [4] Not tested

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ANIMAL MODEL FOR MDI

PART 3

APPENDIX 2

BREATHING RATE PATTERN CHANGES IN GUINEA PIGS SENSITISED TO MDI AND CHALLENGED WITH MDI AEROSOLS

BREATHING RATE AND PATTERN CHANGES IN GUINEA PIGS SENSITISED TO MDI AND CHALLENGED WITH MDI AEROSOLS

Two criteria were used for determination of a positive respiratory response to challenge with MDI aerosols:

a) Breathing Rate
b) Breathing pattern

Breathing Rate

This has been used in all of the work conducted at CTL on respiratory responses of guinea pigs that have been sensitised to pulmonary sensitisers. The classification criteria were developed through experience over a period of 2-3 years. They are based upon individual animal breathing rate measurements during exposure to the sensitiser being compared with rates over an initial control period (which follows a short period of acclimatization to the restraint/plethysmograph tubes). The rates during the control period are normalised to 100% to enable variations from the mean to be scored as percentage changes. The criteria for positive responses have been published and are as follows:

No effect: changes in respiration rate within 71-129% of the normal background rate within the 15 min challenge period.

Moderate response: An increase in respiration rate to 130% or more of the normal background rate within the 15 min challenge period.

Severe response: A rapid decrease in respiration rate to 70% or less of the normal background rate within the 15 min challenge period. This response may be preceded by an increase in respiratory rate.

Copies of respiratory rate plots from a number of groups of animals exposed to MDI aerosols are attached to illustrate these criteria (Figures 1, 9, 14, 21)

Breathing pattern

Breathing patterns were measured on many of our later studies using pressure plethysmography as described in our publication. The equipment was controlled and monitored by a computer which allowed "snapshots" of periods to be saved and printed as a trace of the respiratory pattern which was monitored continually on a monitor by the study operators. Consideration of breathing pattern as positive or negative was made by blind and random reading of coded copies of respiratory pattern traces. Only after scoring was the group and treatment identified and collated by the study investigator and study director.

Copies of respiratory patterns from a number of groups of animals exposed to MDI aerosols are attached to illustrate these criteria.

Normal breathing patterns are generally reasonably smooth and symmetrical, as shown in Figures 3, 10 and 15.

Breathing patterns indicative of a response to challenge with MDI and other respiratory sensitisers vary considerably. Strong responses are easily distinguished, as shown in Figures 8, 18 and 19. Weaker responses range between those shown in Figures 5 & 12/13. The weaker responses are often similar to those seen when animals are exposed to sensory irritants. However, all studies are preceded by preliminary studies to determine the threshold of irritancy of the test material in control animals and challenge exposure concentrations are always maintained below this. The response at challenge which is similar to irritancy but at a lower concentration might indeed be a reflection of an irritant response in an airway which has become hyperreactive due to sensitisation with test material and therefore responds at markedly lower concentration to the normal airway. Further experience from our laboratory and others will help us to interpret these findings more comprehensively.

Figures

Attached figures are of rate patterns and associated breathing patterns from groups treated as follows:

Sensitisation with 0.3% MDI, challenge with different aerosol concentrations

Figures 1-8: 0.3% MDI id. sensitisation, challenge with 28mg/m^3 MDI

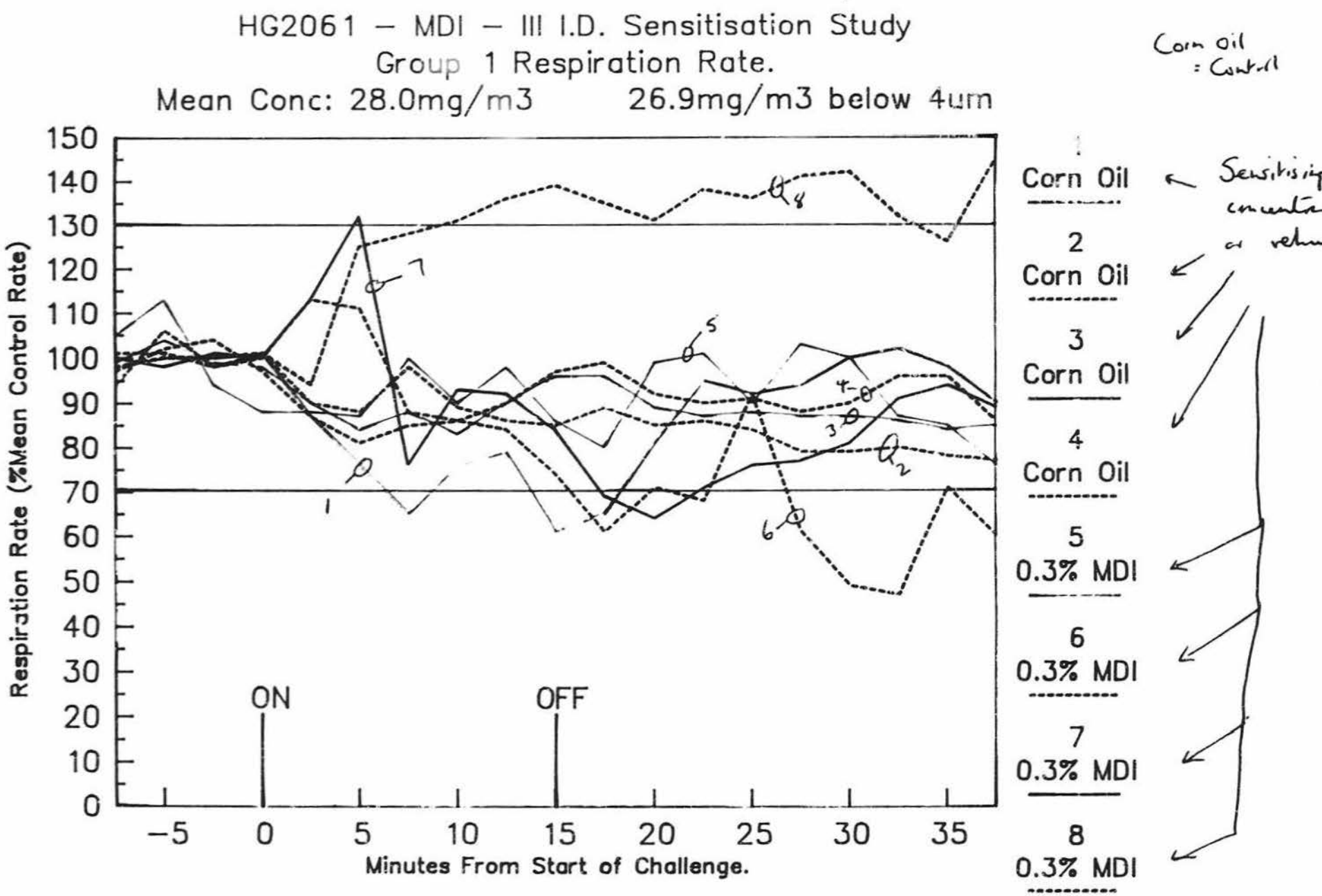
Figures 9-13: 0.3% MDI id. sensitisation, challenge with 2.9mg/m^3 MDI

Sensitisation with different concentrations of MDI (intradermally) and challenge with one aerosol concentration

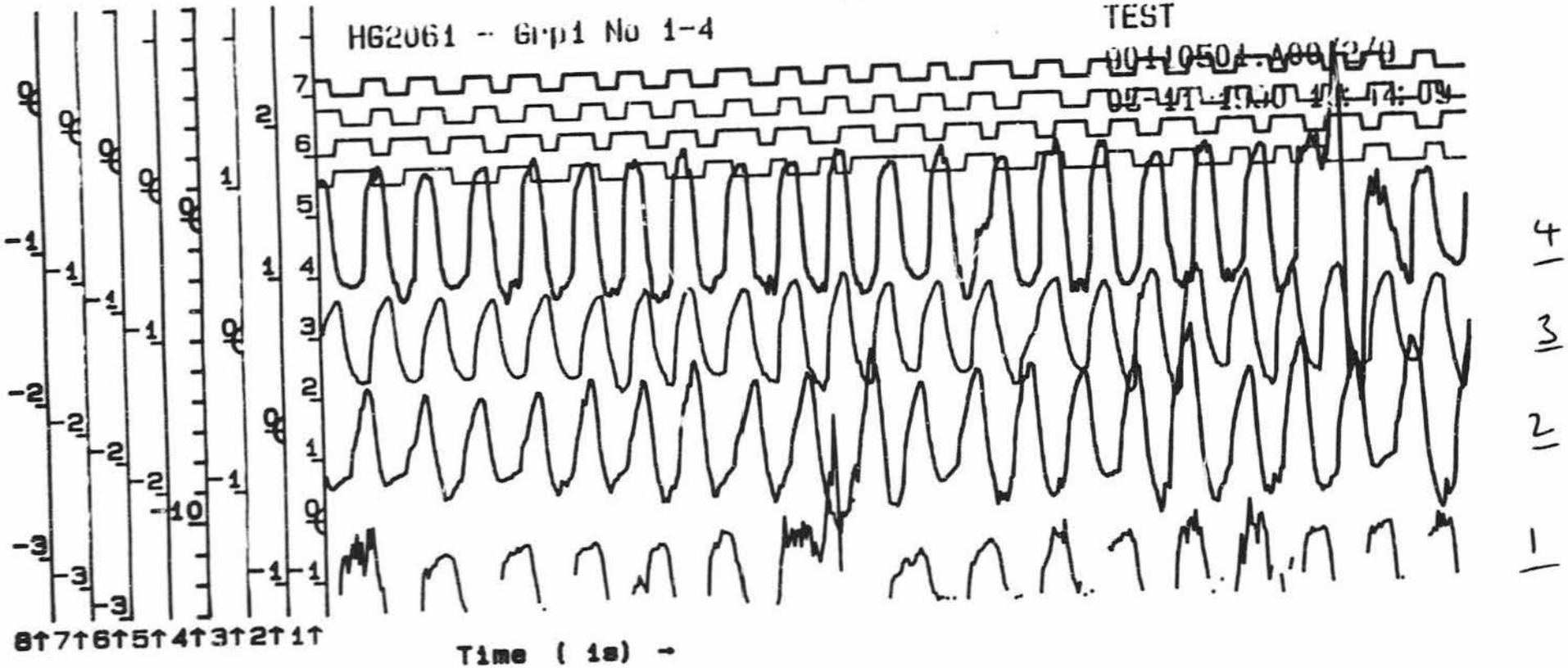
Figures 14-20: 0.3% MDI id. sensitisation, challenge with 35.2mg/m^3 MDI

Figures 21-23: 0.0003% MDI id. sensitisation, challenge with 27.6mg/m^3 MDI

Fig 1


HG2061 – MDI – III I.D. Sensitisation Study
Group 1 Respiration Rate.
Mean Conc: 28.0mg/m3 26.9mg/m3 below 4um
Respiration Rate (%Mean Control Rate)
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ON
OFF
−5
0
5
10
15
20
25
30
35
Minutes From Start of Challenge.
1 Corn Oil
2 Corn Oil
3 Corn Oil
4 Corn Oil
5 0.3% MDI
6 0.3% MDI
7 0.3% MDI
8 0.3% MDI
Corn oil = Control
Sensitising concentration

Control Rewd



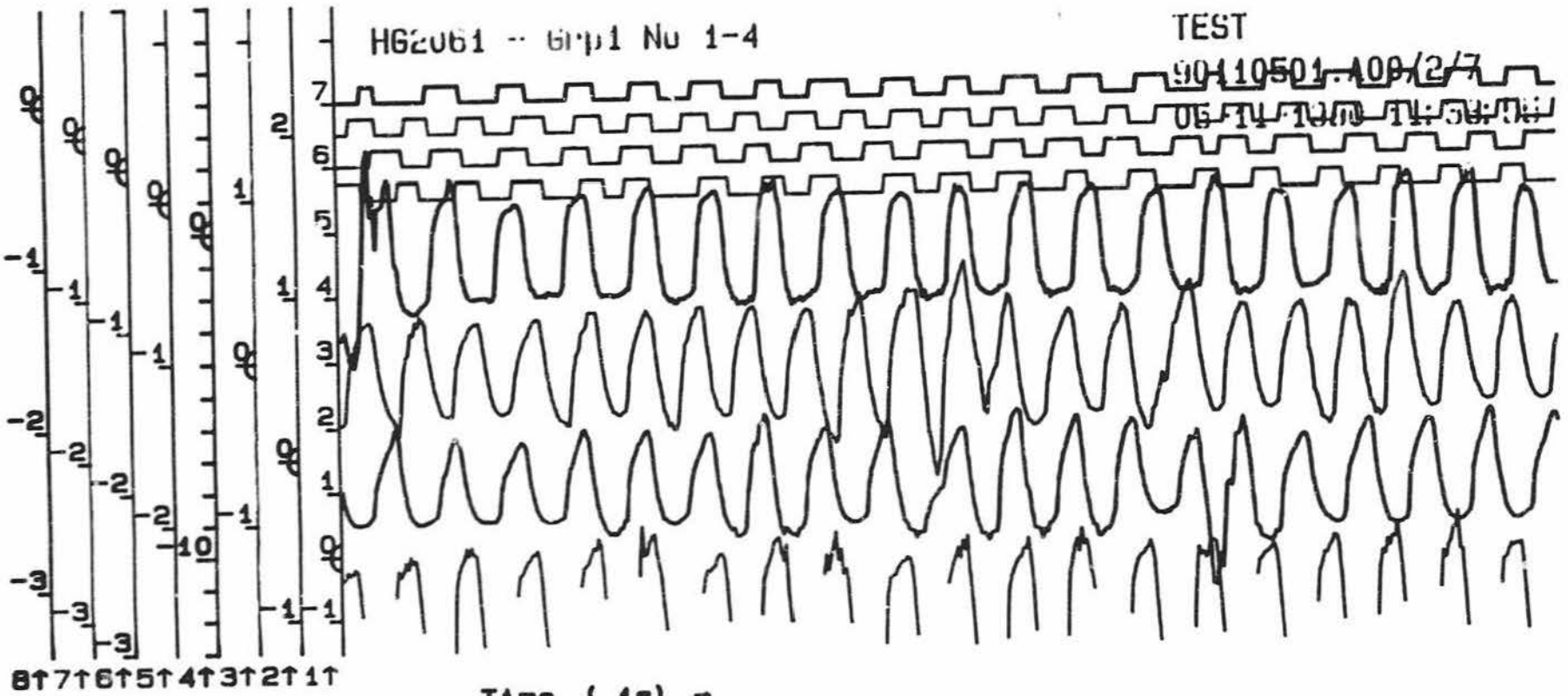
1 -	1 cm H2O	Pressure (Pleth 1)
2 -	10 cm H2O	Pressure (Pleth 2)
3 -	10 cm H2O	Pressure (Pleth 3)
4 -	1 cm H2O	Pressure (Pleth 4)
5 -	10	Direction (Pleth 1)
6 -	10	Direction (Pleth 2)
7 -	10	Direction (Pleth 3)
8 -	10	Direction (Pleth 4)

↑ Animal No in ascending order

48

Exposure Period

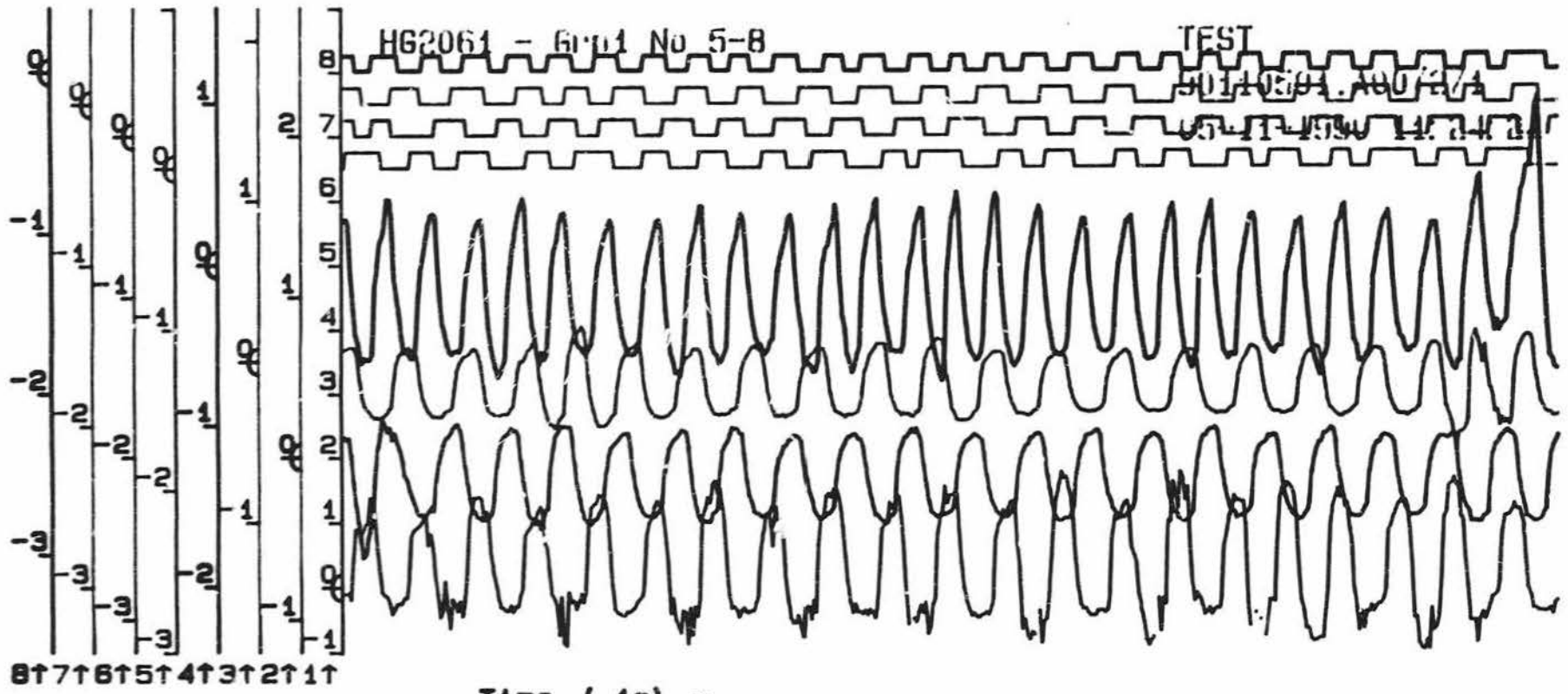
Fig 5



1 -	1 cm H20	Pressure (Pleth 1)
2 -	10 cm H20	Pressure (Pleth 2)
3 -	10 cm H20	Pressure (Pleth 3)
4 -	1 cm H20	Pressure (Pleth 4)
5 -	10	Direction (Pleth 1)
6 -	10	Direction (Pleth 2)
7 -	10	Direction (Pleth 3)
8 -	10	Direction (Pleth 4)

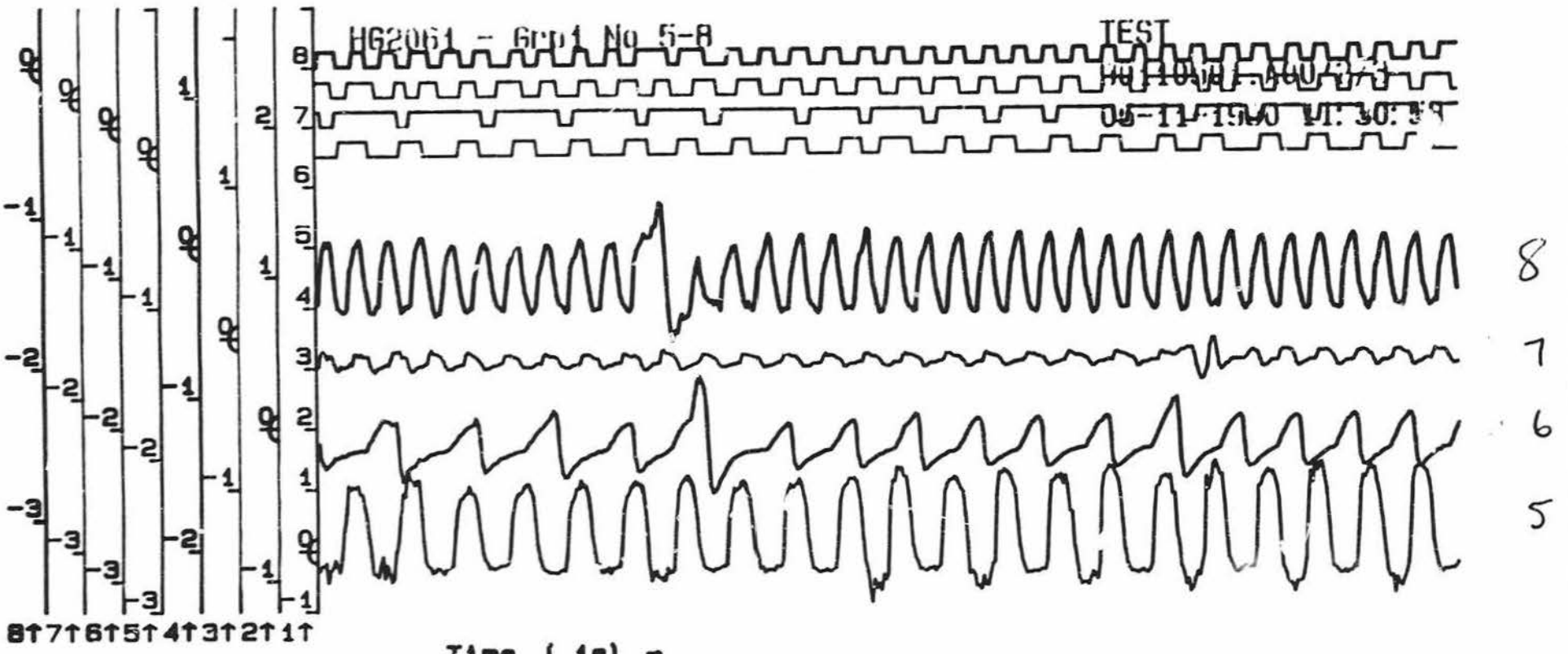
Expiration Period

Fig 4



1 -	1 cm H20	Pressure (Pleth 5)
2 -	10 cm H20	Pressure (Pleth 6)
3 -	10 cm H20	Pressure (Pleth 7)
4 -	10 cm H20	**Pressure (Pleth 8)**
5 -	10	Direction (Pleth 5)
6 -	10	Direction (Pleth 6)
7 -	10	Direction (Pleth 7)
8 -	10	**Direction (Pleth 8)**

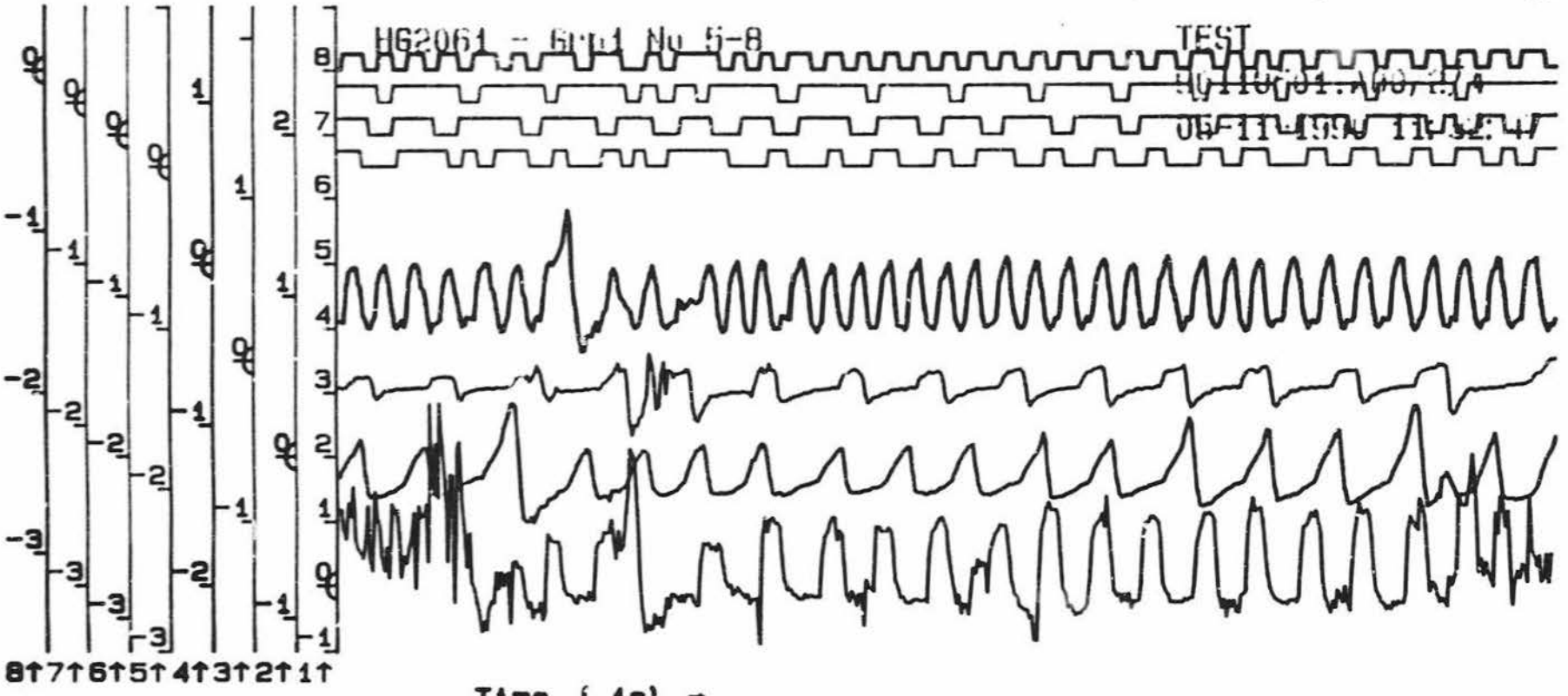
Expiratory trend Fig 3



1 -	1 cm H20	Pressure (Pleth 5)
2 -	10 cm H20	Pressure (Pleth 6)
3 -	10 cm H20	Pressure (Pleth 7)
4 -	10 cm H20	Pressure (Pleth 8)
5 -	10	Direction (Pleth 5)
6 -	10	Direction (Pleth 6)
7 -	10	Direction (Pleth 7)
8 -	10	Direction (Pleth 8)

51

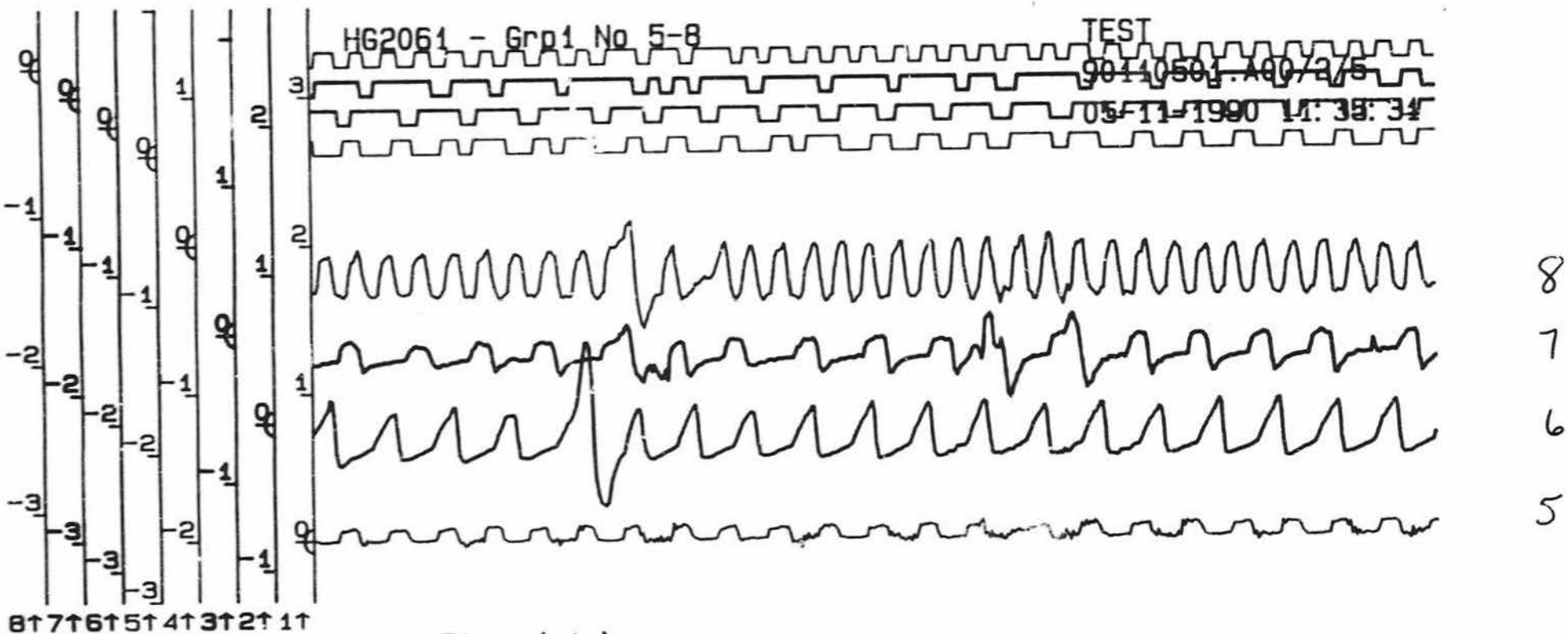
Exposure Record Fig 6



1 -	1 cm H20	Pressure (Pleth 5)
2 -	10 cm H20	Pressure (Pleth 6)
3 -	10 cm H20	Pressure (Pleth 7)
4 -	**10 cm H20**	**Pressure (Pleth 8)**
5 -	10	Direction (Pleth 5)
6 -	10	Direction (Pleth 6)
7 -	10	Direction (Pleth 7)
8 -	**10**	**Direction (Pleth 8)**

52

Expiration Period Fig 1.

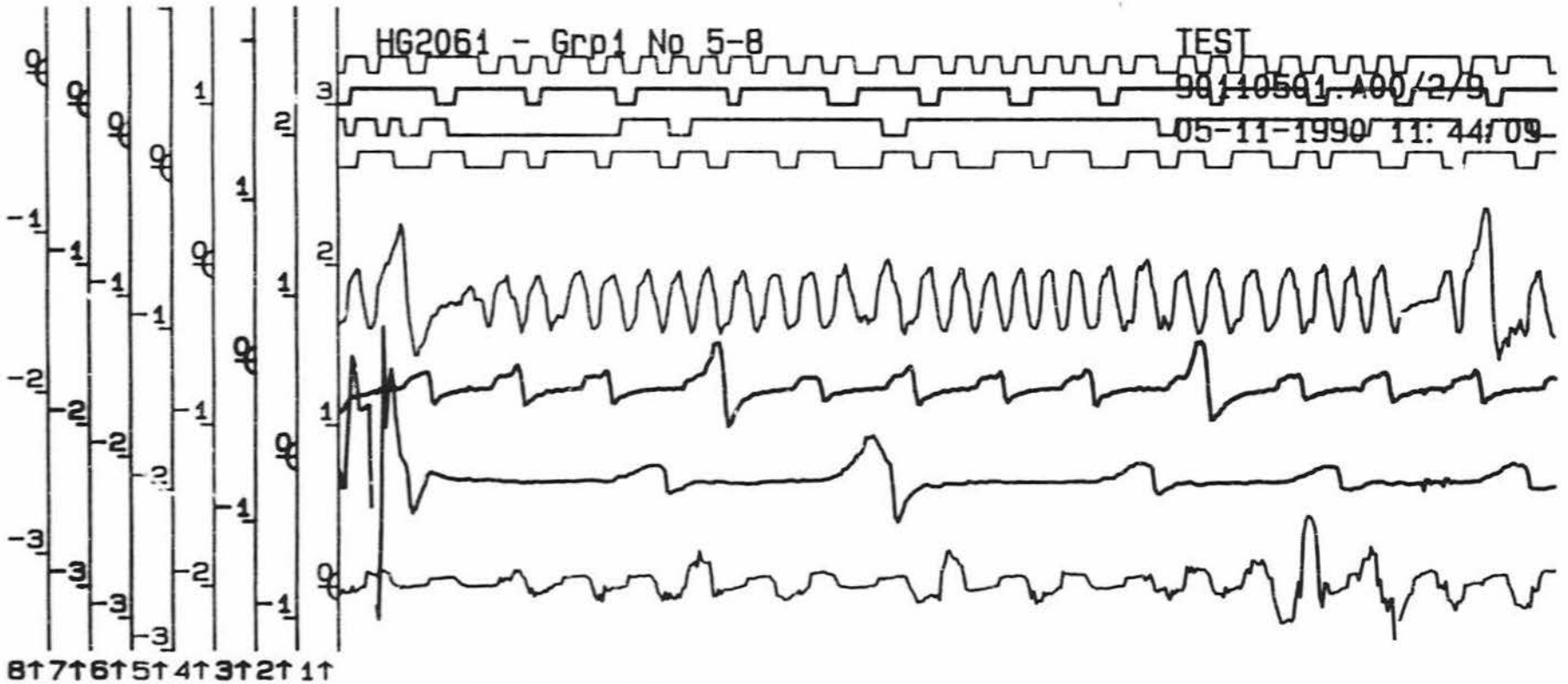


1 -	10 cm H2O	Pressure (Pleth 5)
2 -	10 cm H2O	Pressure (Pleth 6)
3 -	10 cm H2O	Pressure (Pleth 7)
4 -	10 cm H2O	Pressure (Pleth 8)
5 -	10	Direction (Pleth 5)
6 -	10	Direction (Pleth 6)
7 -	10	Direction (Pleth 7)
8 -	10	Direction (Pleth 8)

53

Exposure permit

Fig 8

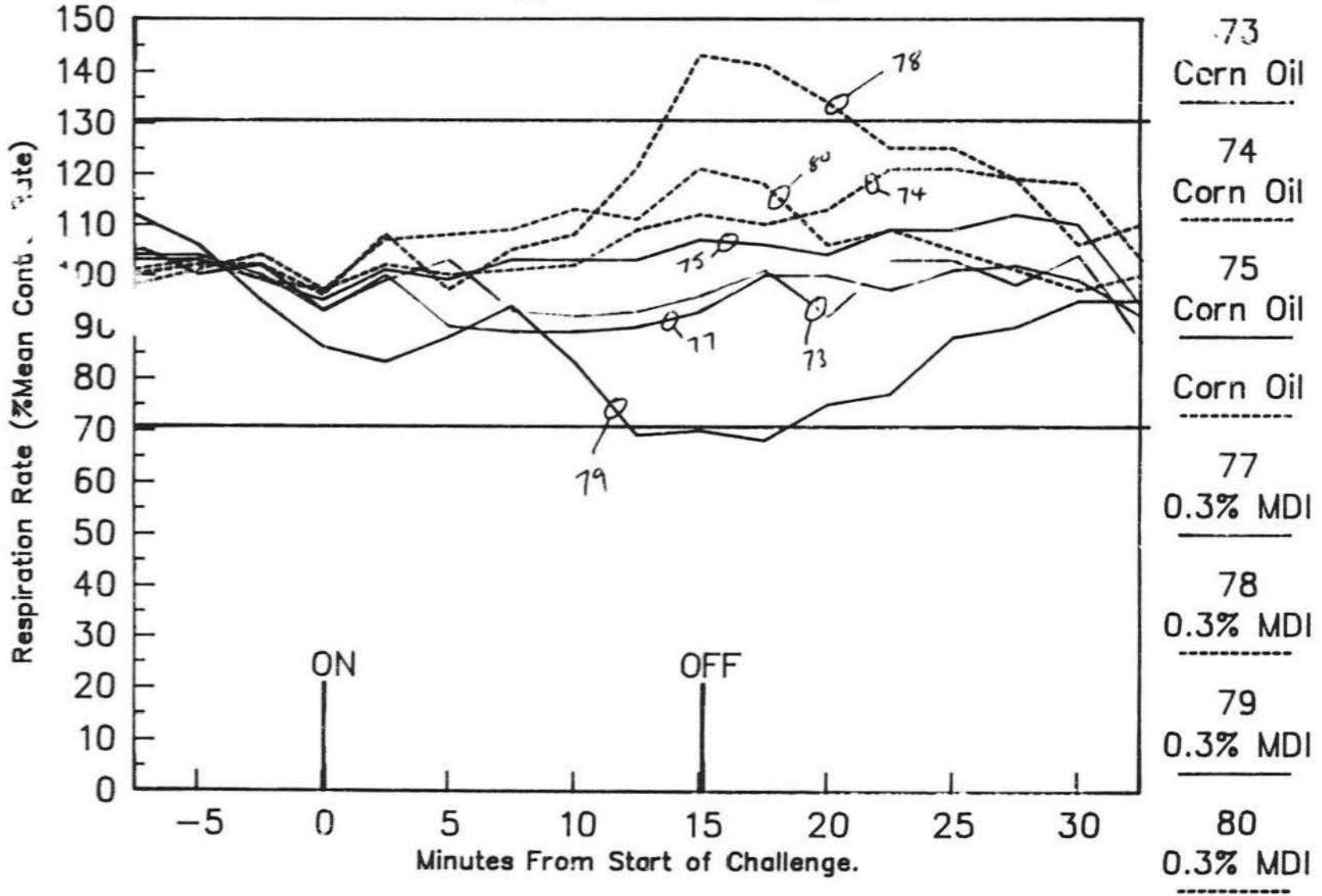


Time (1s) →

1 -	10 cm H20	Pressure (Pleth 5)
2 -	10 cm H20	Pressure (Pleth 6)
3 -	10 cm H20	Pressure (Pleth 7)
4 -	10 cm H20	Pressure (Pleth 8)
5 -	10	Direction (Pleth 5)
6 -	10	Direction (Pleth 6)
7 -	10	Direction (Pleth 7)
8 -	10	Direction (Pleth 8)

Fig 7
2915

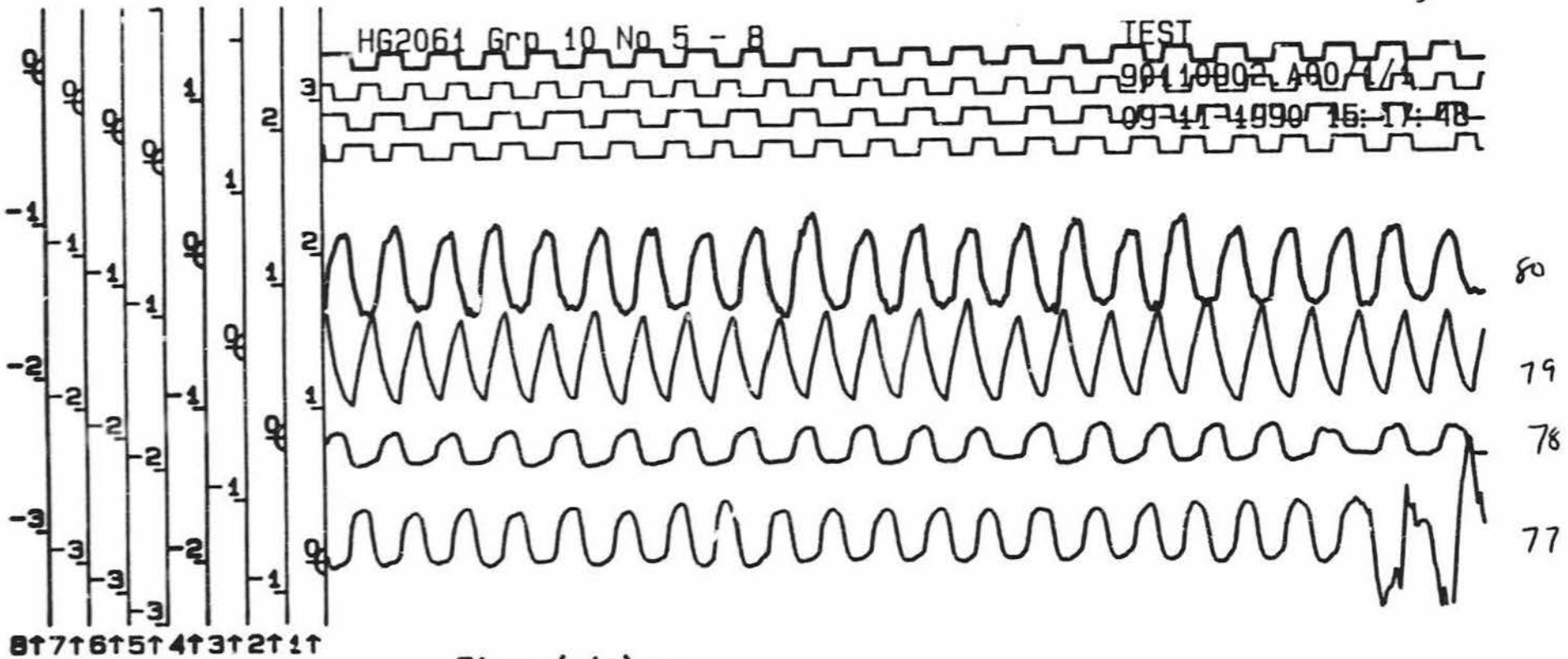
HG2061 – MDI – III I.D. Sensitisation Study
Group 10 Respiration Rate.
Mean Conc: 2.9mg/m3 2.5mg/m3 below 4um



55

Control Tension

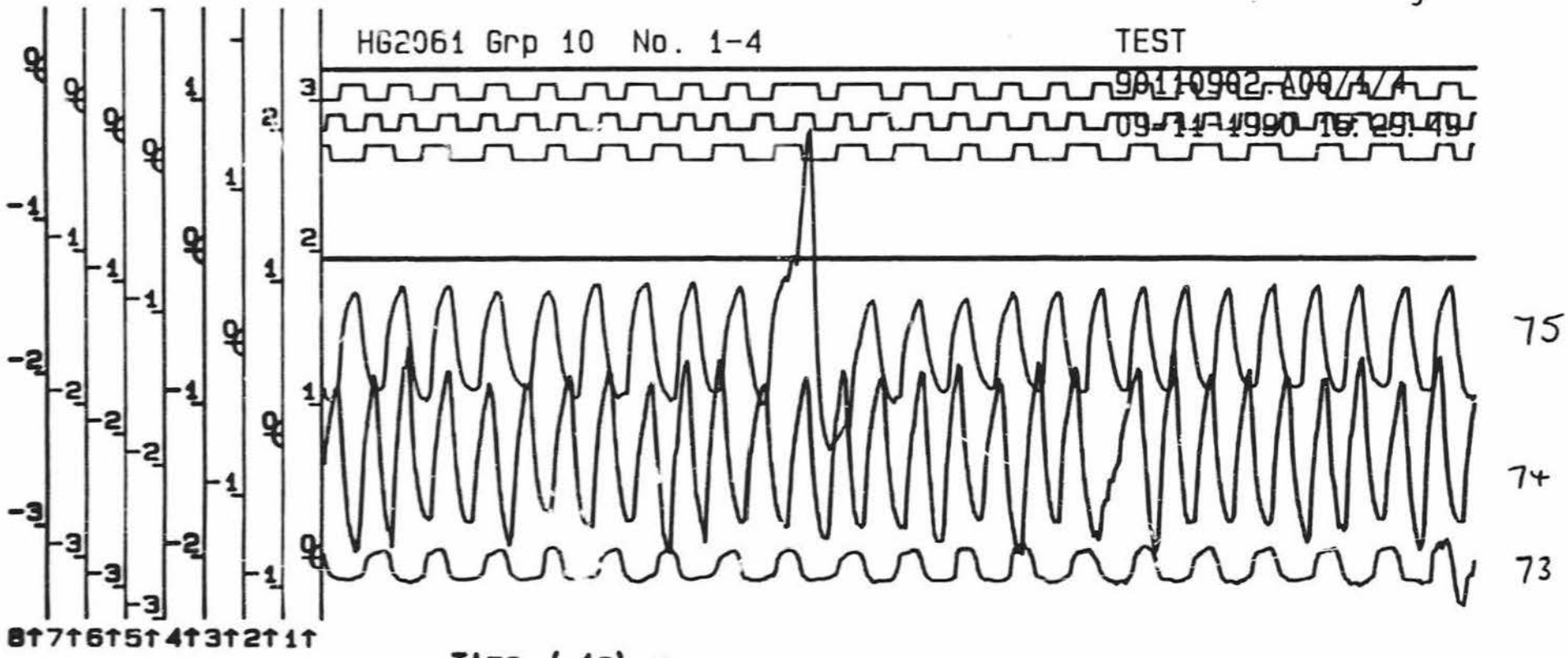
Fig 10



1 -	10 cm H2O	Pressure (Pleth 5)
2 -	10 cm H2O	Pressure (Pleth 6)
3 -	10 cm H2O	Pressure (Pleth 7)
4 -	**10 cm H2O**	**Pressure (Pleth 8)**
5 -	10	Direction (Pleth 5)
6 -	10	Direction (Pleth 6)
7 -	10	Direction (Pleth ..)
8 -	**10**	**Direction (Pleth 8)**

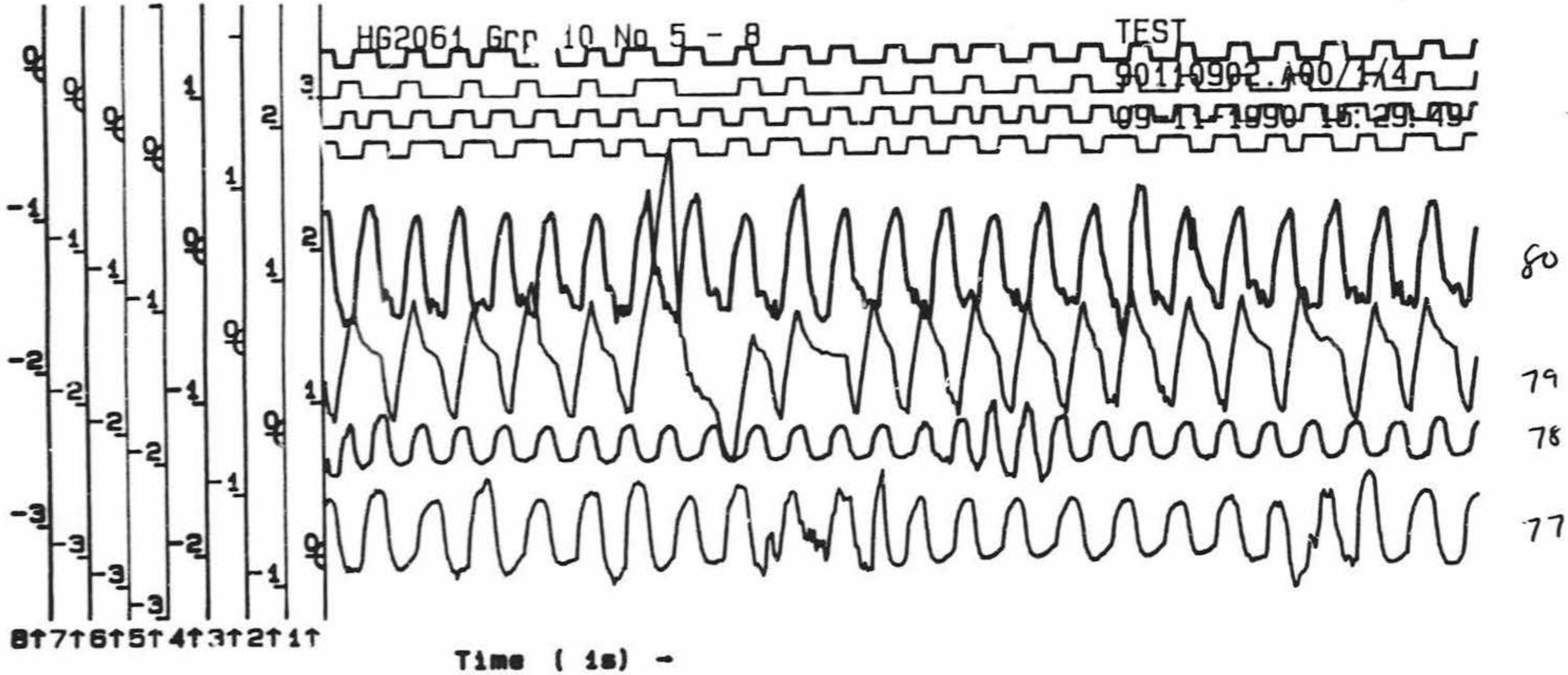
SL

Fig 11 2 713



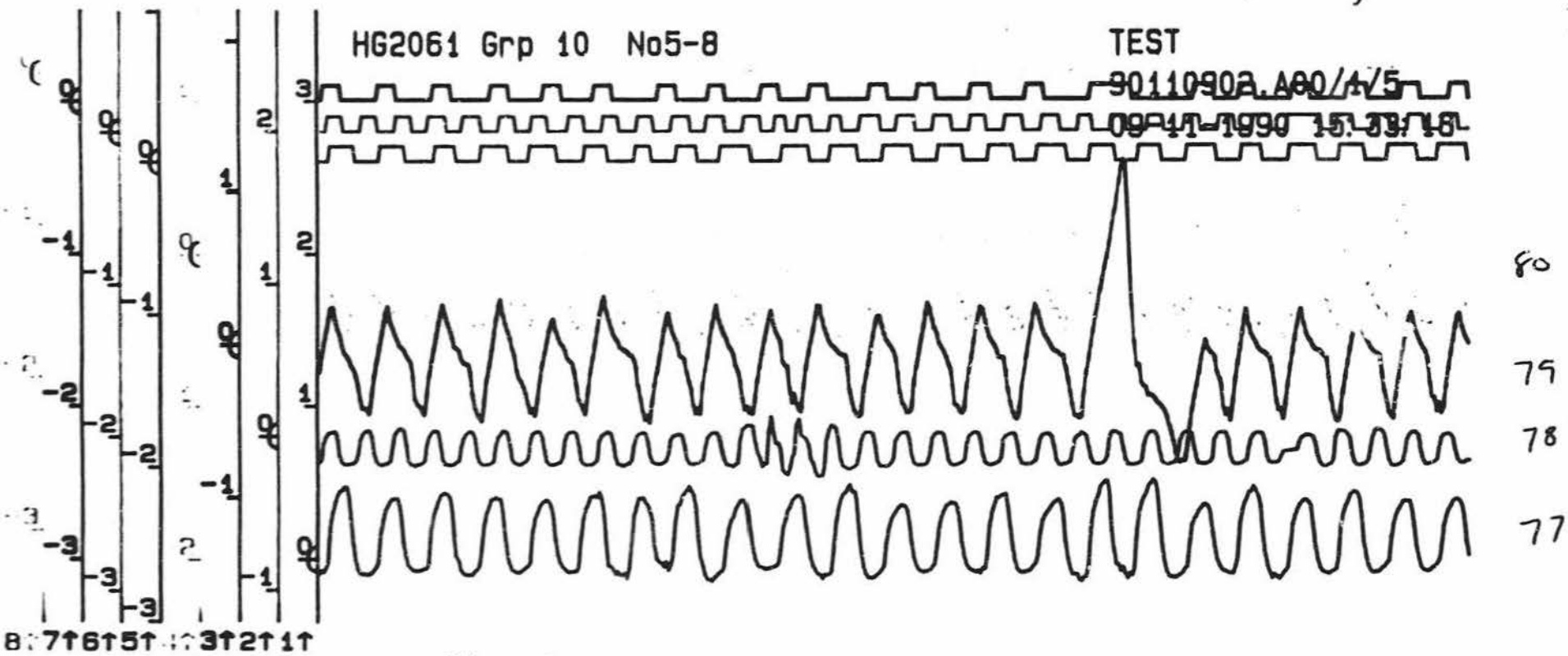
1 -	10 cm H2O	Pressure (Pleth 1)
2 -	10 cm H2O	Pressure (Pleth 2)
3 -	10 cm H2O	Pressure (Pleth 3)
4 -	**10 cm H2O**	**Pressure (Pleth 4)**
5 -	10	Direction (Pleth 1)
6 -	10	Direction (Pleth 2)
7 -	10	Direction (Pleth 3)
8 -	**10**	**Direction (Pleth 4)**

Fig 12 341 3



1 - 10 cm H2O Pressure (Pleth 5)
2 - 10 cm H2O Pressure (Pleth 6)
3 - 10 cm H2O Pressure (Pleth 7)
4 - 10 cm H2O Pressure (Pleth 8)
5 - 10 Direction (Pleth 5)
6 - 10 Direction (Pleth 6)
7 - 10 Direction (Pleth 7)
8 - 10 Direction (Pleth 8)

Expiration period fig 13 4st 4



1 - 10 cm H20 Pressure (Pleth 5)
2 - 10 cm H20 Pressure (Pleth 6)
3 - 10 cm H20 Pressure (Pleth 7)

5 - 10 Direction (Pleth 5)
6 - 10 Direction (Pleth 6)
7 - 10 Direction (Pleth 7)
8 - 10 Direction (Pleth 8)

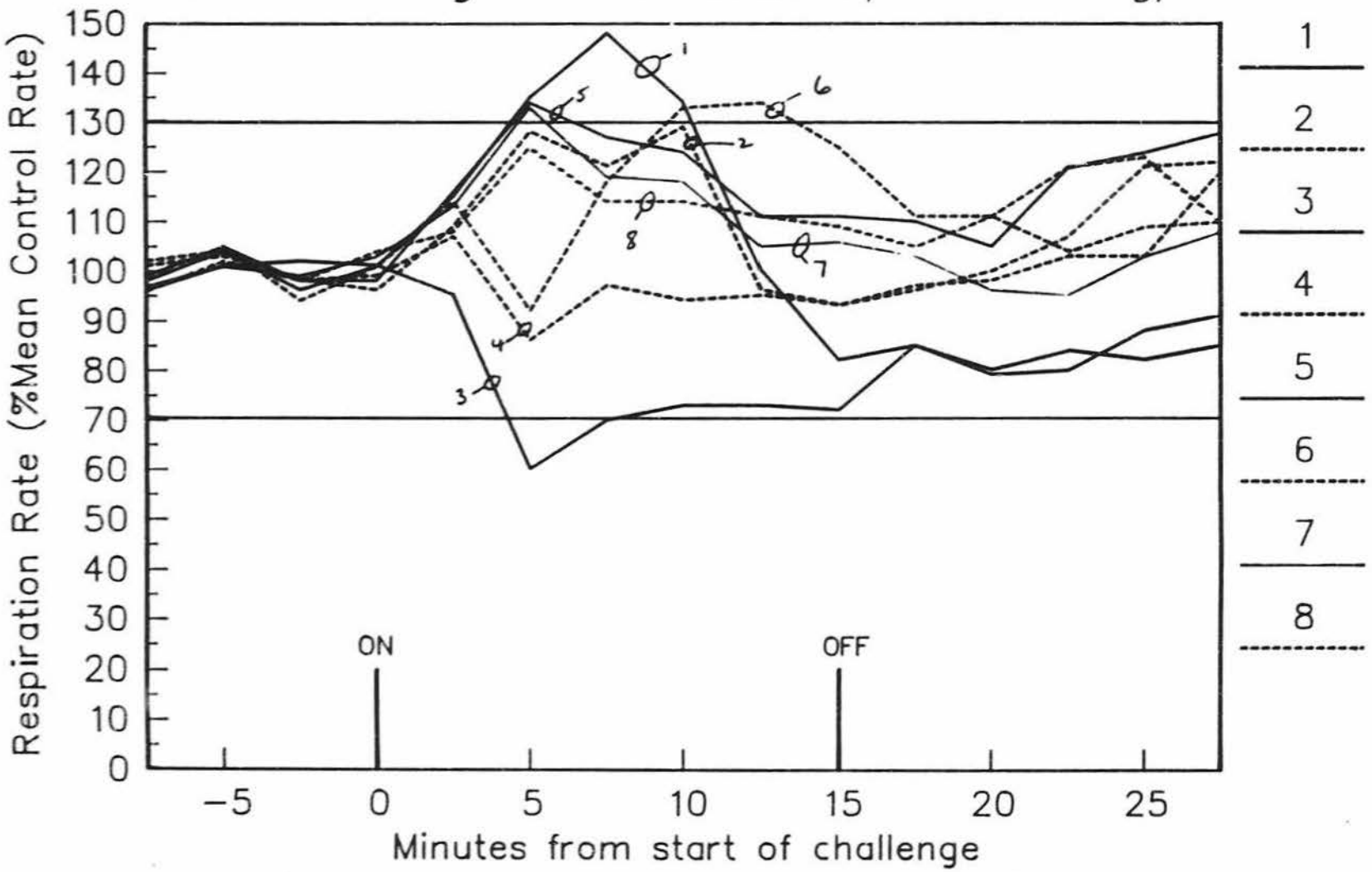
59

Fig 14

HG2069 MDI – III I.D. Sensitisation Study

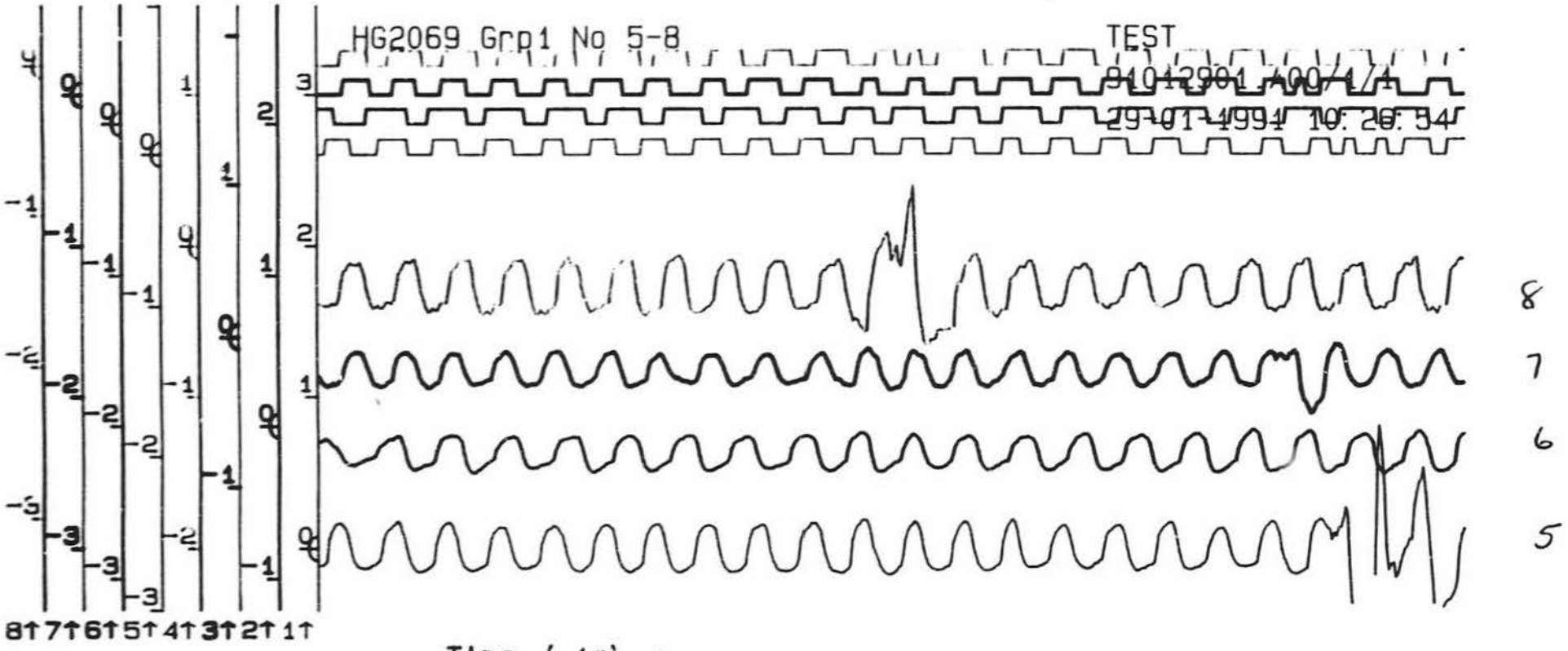
Group 1 – 0.3%w/w I.D. Sens.

Mean Challenge Conc 35.2 +/– 1.2 mg/m3



Control period

Fig 15

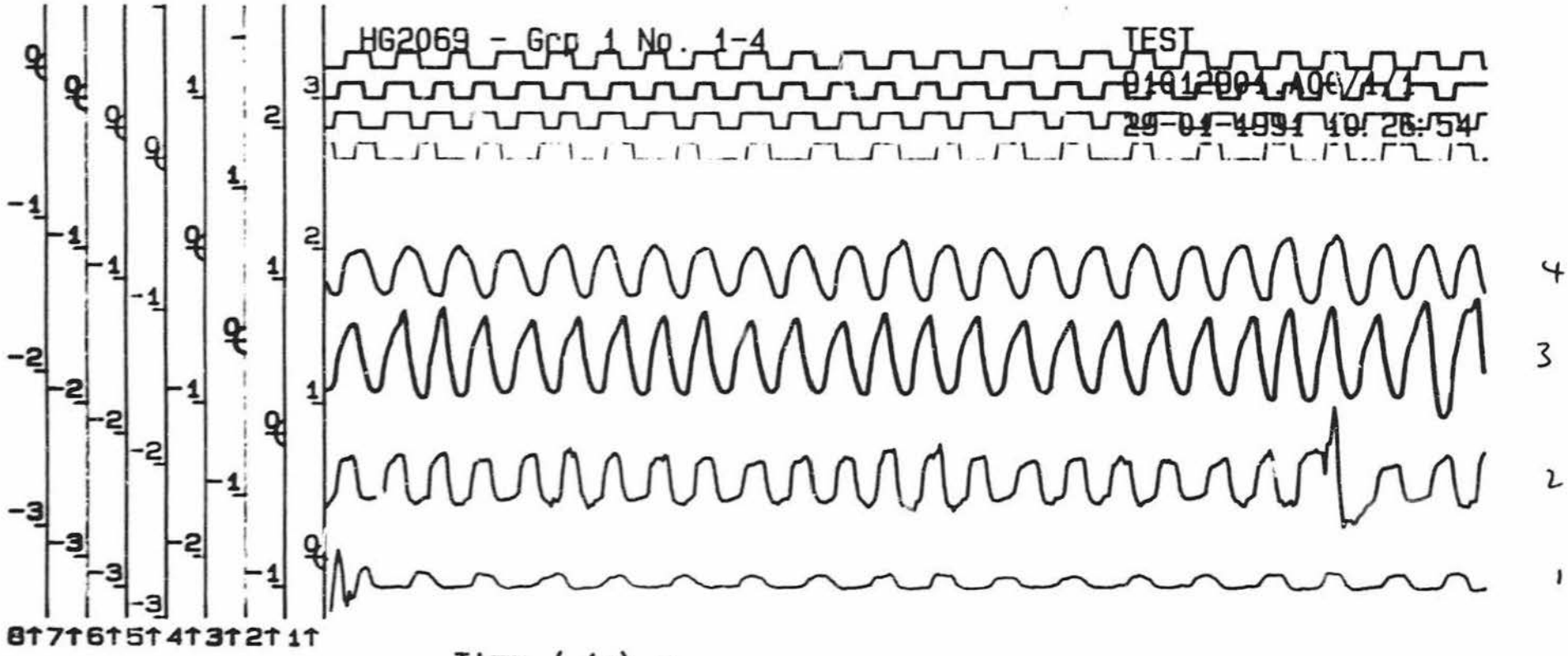


1 -	10 cm H2O	Pressure (Pleth 5)
2 -	10 cm H2O	Pressure (Pleth 6)
3 -	10 cm H2O	Pressure (Pleth 7)
4 -	10 cm H2O	Pressure (Pleth 8)
5 -	10	Direction (Pleth 5)
6 -	10	Direction (Pleth 6)
7 -	10	Direction (Pleth 7)
8 -	10	Direction (Pleth 8)

5

Control period

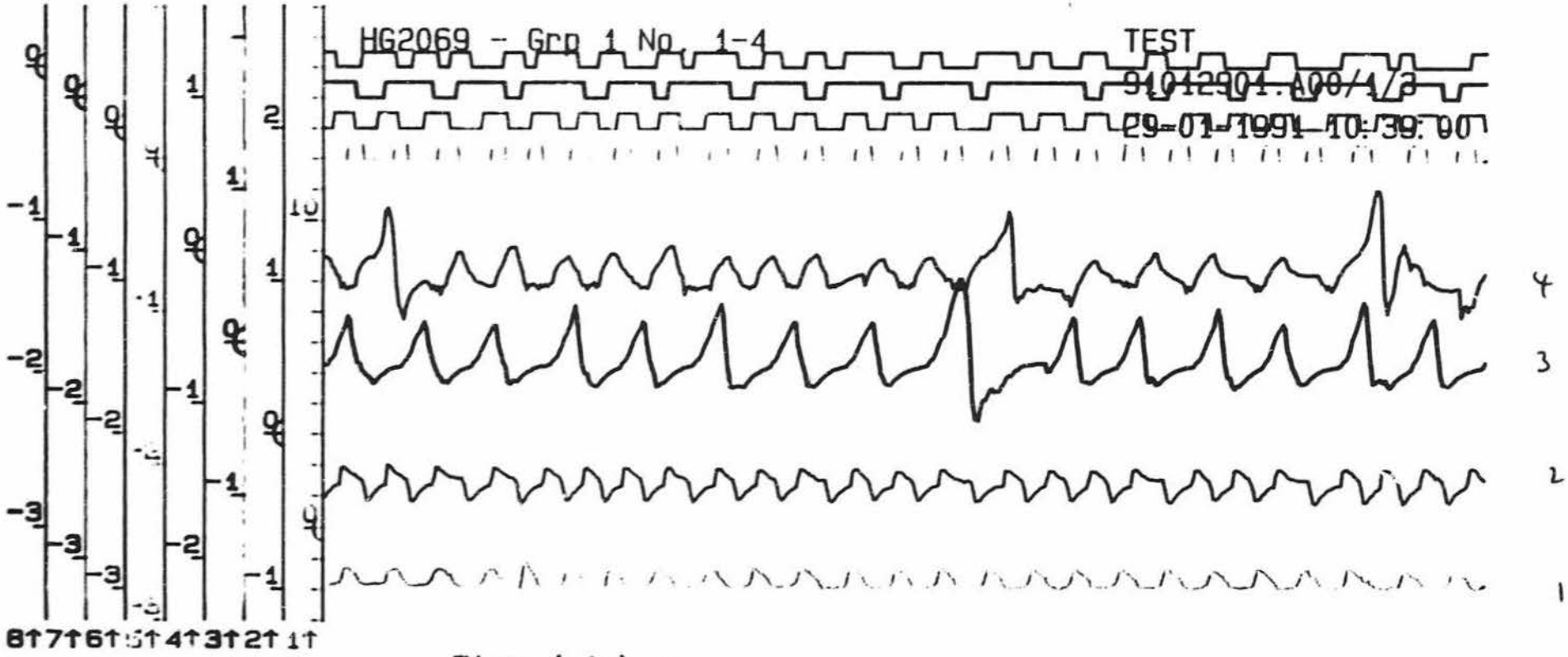
Fig 16



1 -	) cm H20	Pressure (Pleth 1)
2 -	10 cm H20	Pressure (Pleth 2)
3 -	10 cm H20	Pressure (Pleth 3)
4 -	10 cm H20	Pressure (Pleth 4)
5 -	10	Direction (Pleth 1)
6 -	10	Direction (Pleth 2)
7 -	10	Direction (Pleth 3)
8 -	10	Direction (Pleth 4)

Exposure.

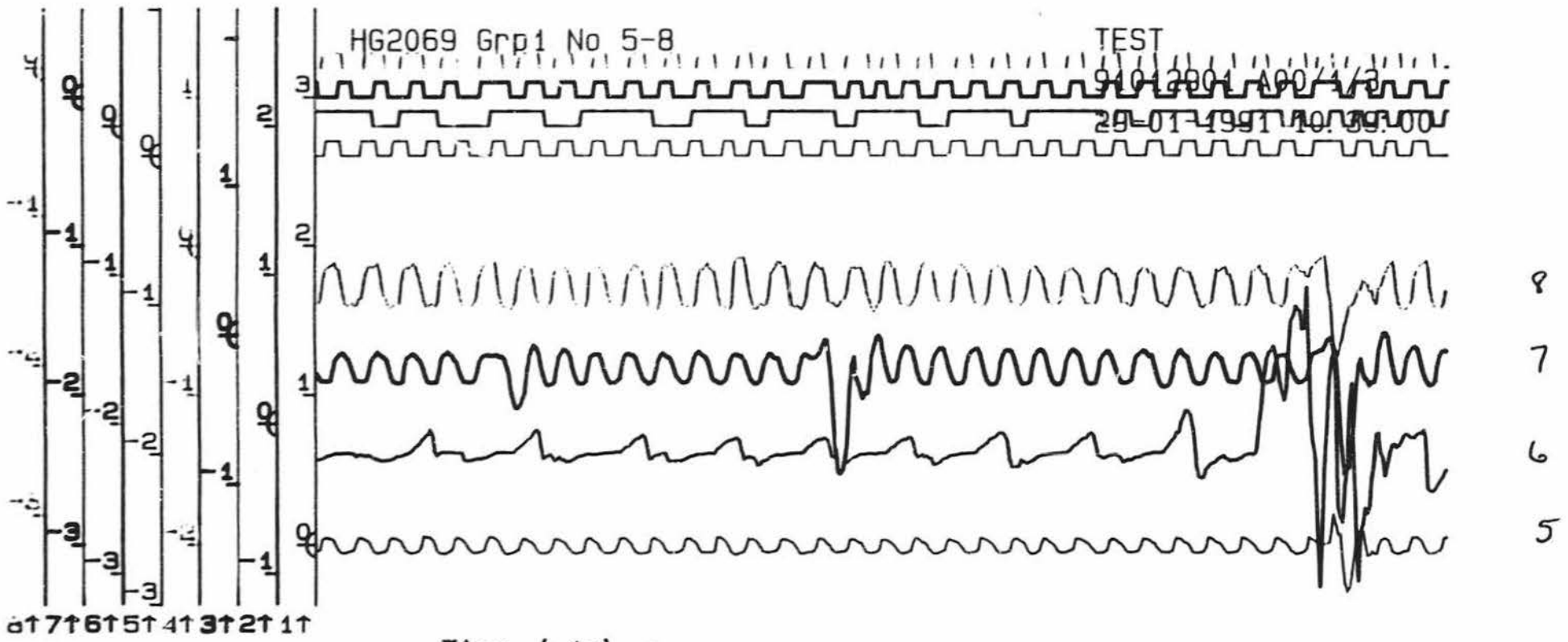
Fig 17



1 -	1 cm H2O	Pressure (Pleth 1)
2 -	10 cm H2O	Pressure (Pleth 2)
3 -	10 cm H2O	Pressure (Pleth 3)
4 -	10 cm H2O	Pressure (Pleth 4)
5 -	10	Direction (Pleth 1)
6 -	10	Direction (Pleth 2)
7 -	10	Direction (Pleth 3)
8 -	10	Direction (Pleth 4)

Exposure

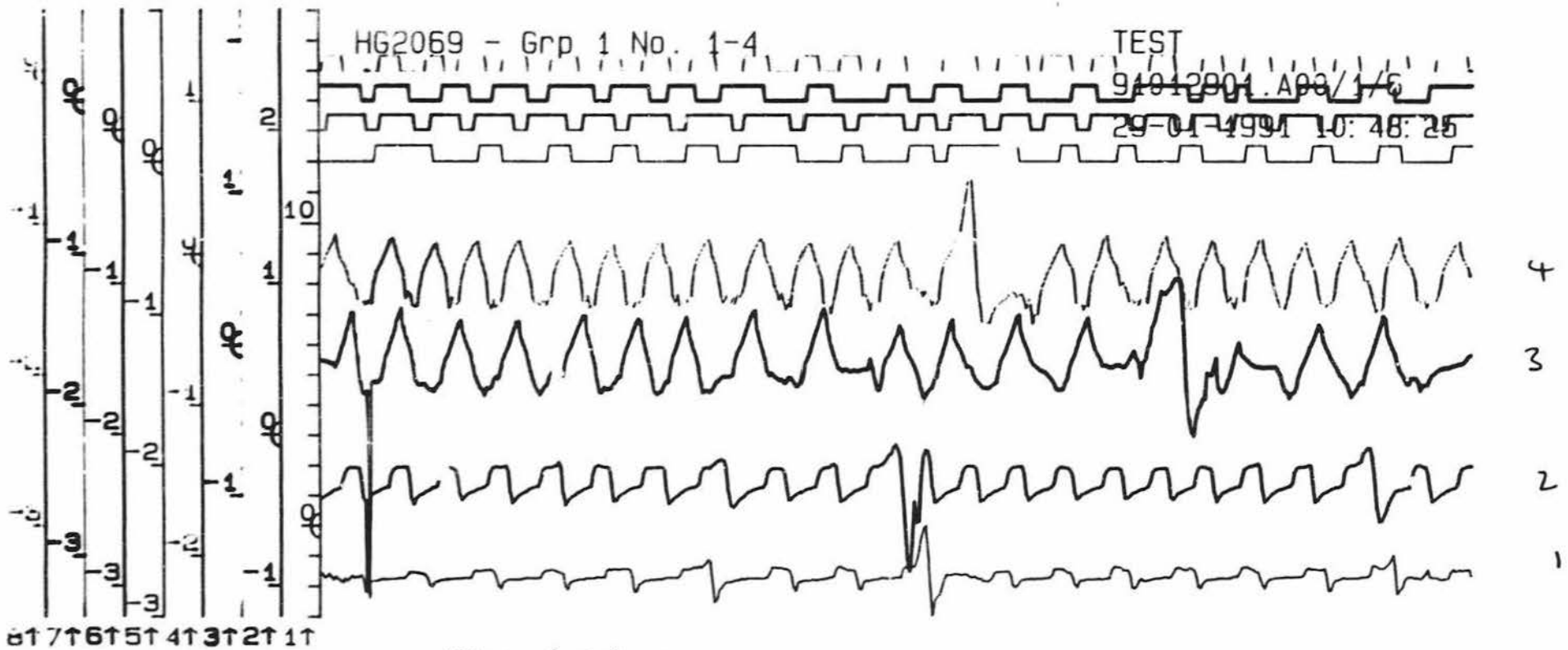
Fig 18



1 -	10 cm H20	Pressure (Pleth 5)
2 -	10 cm H20	Pressure (Pleth 6)
3 -	10 cm H20	Pressure (Pleth 7)
4 -	10 cm H20	Pressure (Pleth 8)
5 -	10	Direction (Pleth 5)
6 -	10	Direction (Pleth 6)
7 -	10	Direction (Pleth 7)
8 -	10	Direction (Pleth 8)

Ex Pleth

Fig 19.



1 -	1 cm H20	Pressure (Pleth 1)
2 -	10 cm H20	Pressure (Pleth 2)
3 -	10 cm H20	Pressure (Pleth 3)
4 -	10 cm H20	Pressure (Pleth 4)
5 -	10	Direction (Pleth 1)
6 -	10	Direction (Pleth 2)
7 -	10	Direction (Pleth 3)
8 -	10	Direction (Pleth 4)

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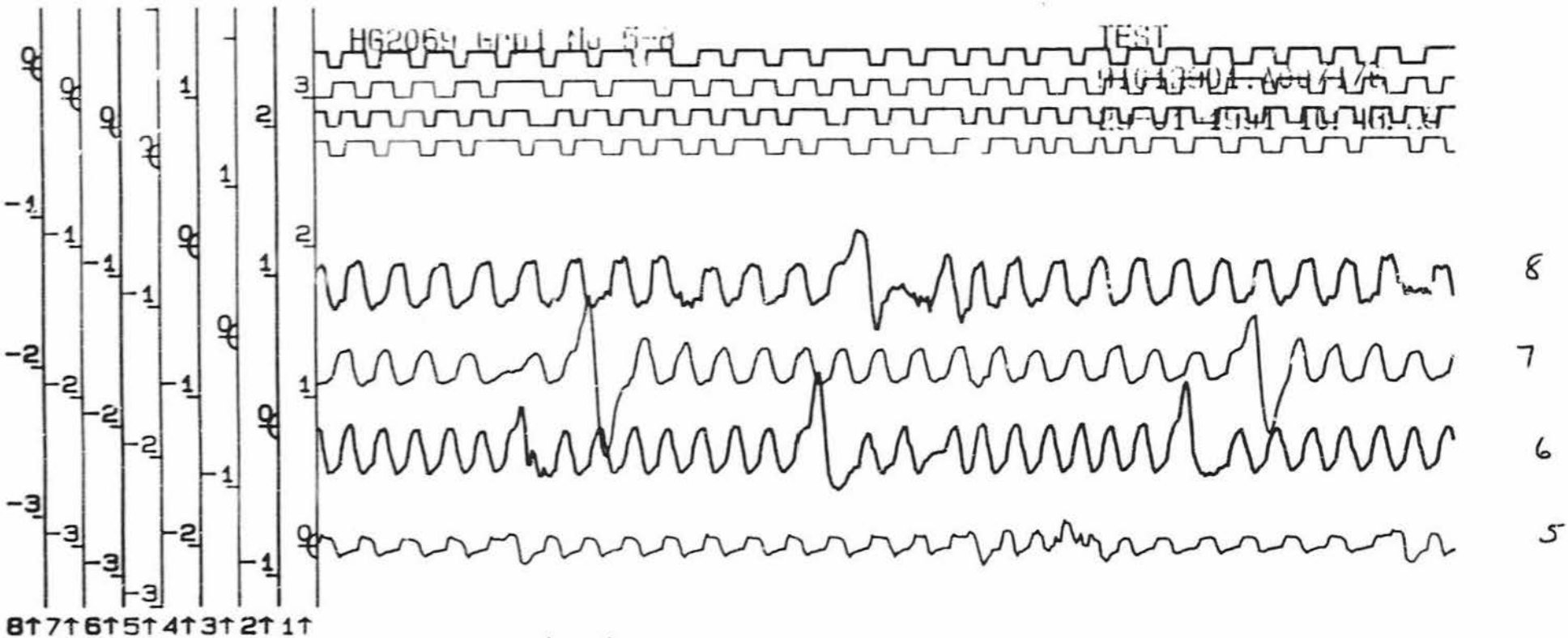
Expiration

Fig 20



1 -	10 cm H20	Pressure (Pleth 5)
2 -	10 cm H20	Pressure (Pleth 6)
3 -	10 cm H20	Pressure (Pleth 7)
4 -	10 cm H20	Pressure (Pleth 8)
5 -	10	Direction (Pleth 5)
6 -	10	Direction (Pleth 6)
7 -	10	Direction (Pleth 7)
8 -	10	Direction (Pleth 8)

Fig 21

HG2069 MDI – III I.D. Sensitisation Study
Group 4 – 0.0003%w/w I.D. Sens.
Mean Challenge Conc 27.6 +/– 8.8 mg/m3

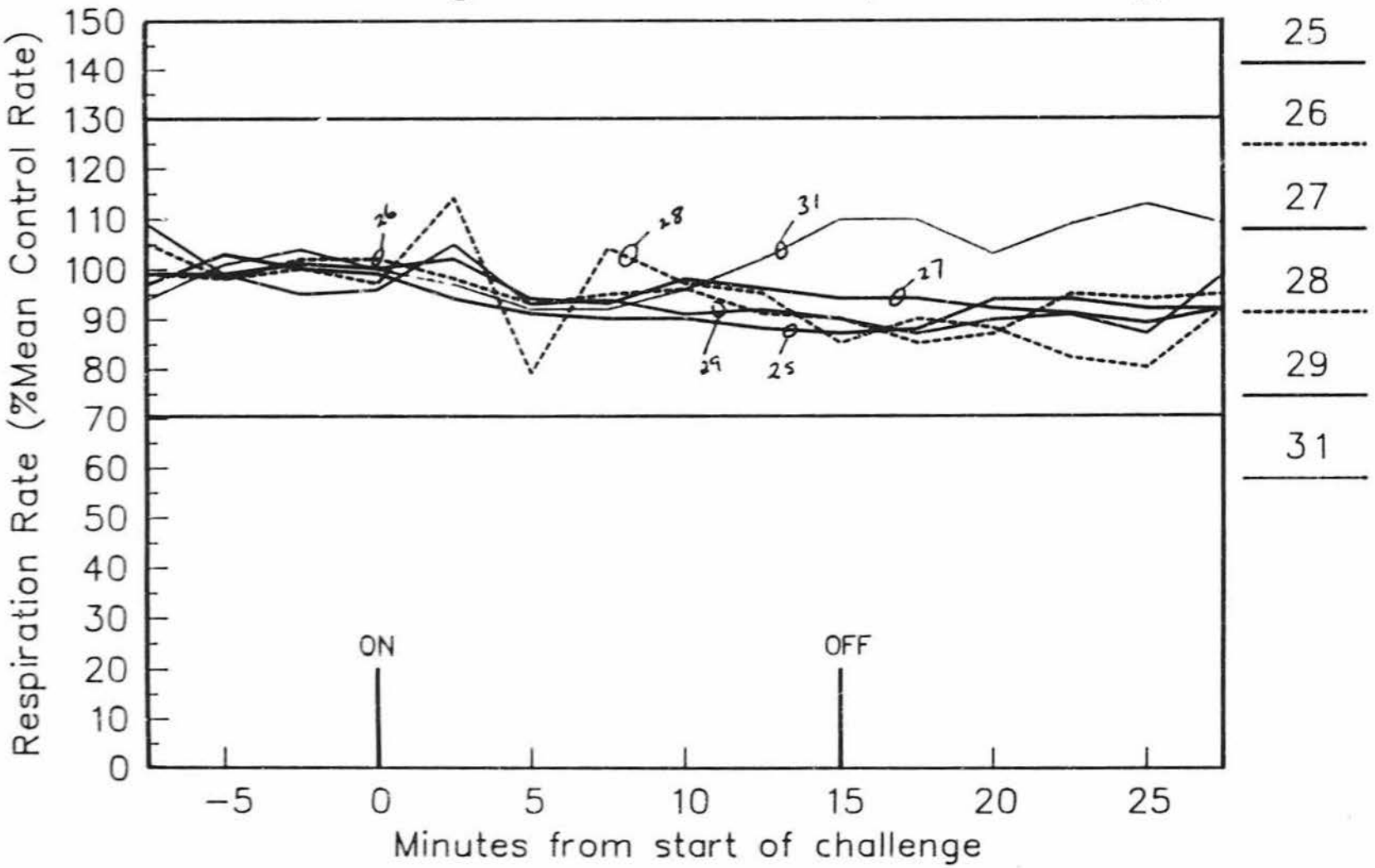
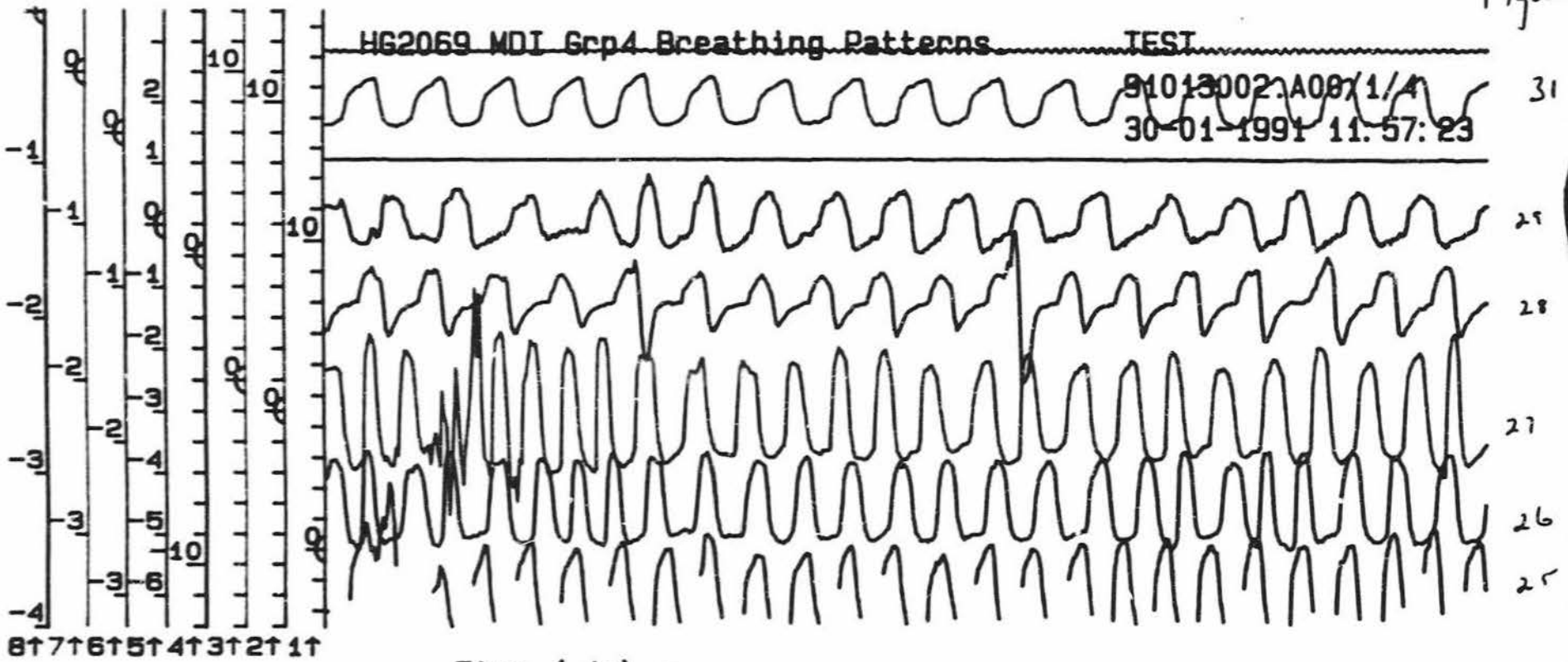
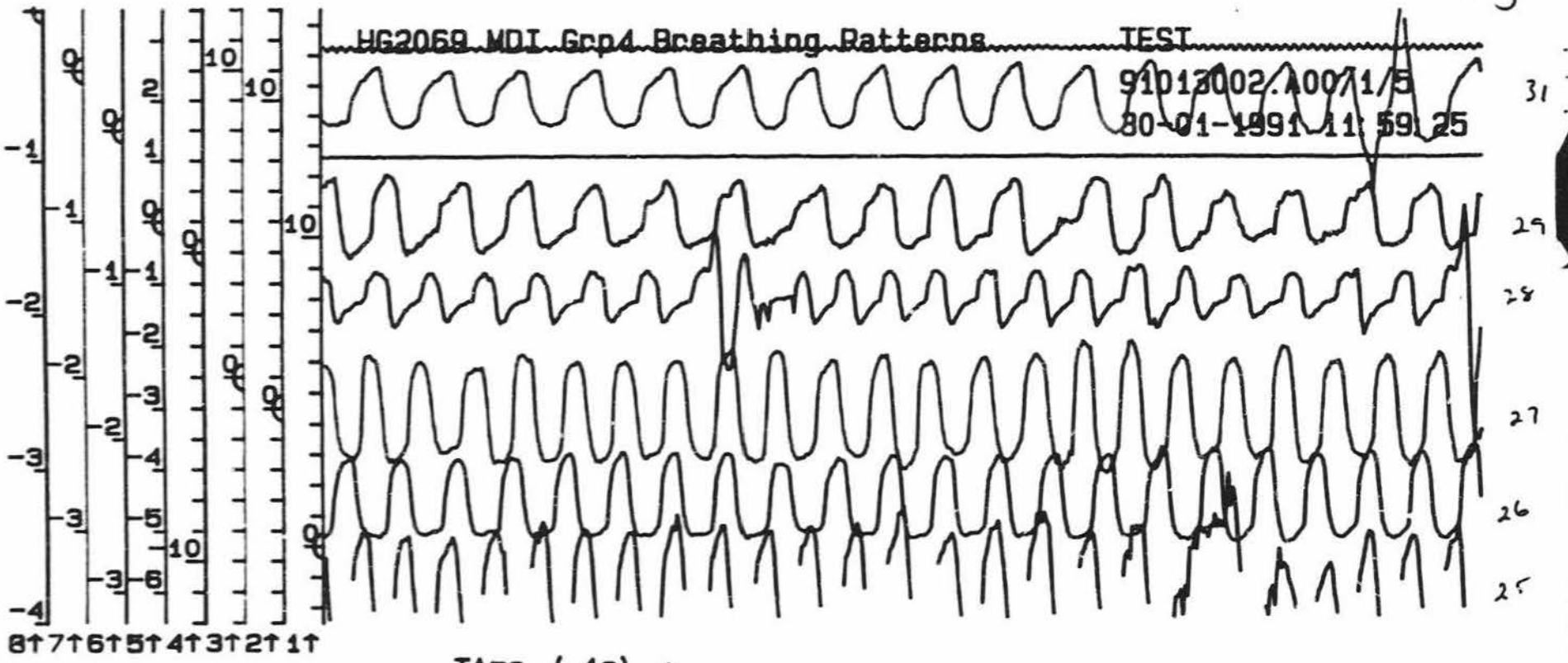


Fig 22



1 -	1 cm H20	Pressure (Pleth 1)
2 -	1 cm H20	Pressure (Pleth 2)
3 -	1 cm H20	Pressure (Pleth 3)
4 -	1 cm H20	Pressure (Pleth 4)
5 -	1 cm H20	Pressure (Pleth 5)
6 -	10 cm H20	Pressure (Pleth 6)
7 -	10 cm H20	Pressure (Pleth 7)
8 -	10 cm H20	Pressure (Pleth 8)

Fig 15



1 -	1 cm H20	Pressure (Pleth 1)
2 -	1 cm H20	Pressure (Pleth 2)
3 -	1 cm H20	Pressure (Pleth 3)
4 -	1 cm H20	Pressure (Pleth 4)
5 -	1 cm H20	Pressure (Pleth 5)
6 -	10 cm H20	Pressure (Pleth 6)
7 -	10 cm H20	Pressure (Pleth 7)
8 -	10 cm H20	Pressure (Pleth 8)

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